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Preface

K. H. Mills and D. W. Schindler

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This is the fourth issue of the *Canadian Journal of Fisheries and Aquatic Sciences* based entirely on research conducted at the Experimental Lakes Area (ELA) in northwestern Ontario. The first ELA issue of this journal contained 14 papers that characterized background environmental data at the ELA (J. Fish Res. Board Can. 28 (2)). The second issue (J. Fish. Res. Board Can. 30(10)) contained 13 papers that described early results of the first whole-lake eutrophication experiment in Lake 227 and development of methods to study the chemistry and primary productivity of ELA lakes. The third issue (Can J. Fish Aquat. Sci. 37(3)) contained 26 papers that described results for five whole-lake experiments (two eutrophication, a metal radiotracer, a radiocarbon, and an acidification), process oriented research, long-term monitoring research, and additional methods development. This fourth issue contains 26 papers that describe results for three whole-lake experiments (two eutrophication, one acidification), process oriented acidification research, a cadmium experiment, a wetland acidification experiment, and methods development papers.

Our original intent for this issue was to collate papers describing results of only two whole-lake experiments in Lakes 226 and 223. The scope of papers in this supplement rapidly expanded beyond these two experiments. This reflects the continued desire of many ELA researchers to have their papers published together. Although new whole-lake experiments have started through the years at the ELA, many of the original eutrophication experiments have continued. Examples of this are the Lake 227 experiment (see paper by Schindler et al. 1987), started in 1969, and the Lake 226 experiment (see papers by Findlay and Kasian 1987; Shearer et al. 1987b; Mills and Chalanchuk 1987; and Hesslein 1987), started in 1973. The value of continuing these whole-lake studies can be seen clearly in results presented in the above papers. Changes continue to occur in each experiment. Even after 15 yr of nitrogen and phosphorus additions to Lake 227, the nitrogen budget for the lake has not reached a new equilibrium, and lack of sediment mixing since 1974 suggests that surface sediments may now be rapidly approaching phosphorus equilibrium (Schindler et al. 1987).

Whole-lake experiments often have objectives that are too short-term or are terminated because funding stops. We have often continued whole-lake experiments after the short-term objectives were completed or funding reduced. This has usually involved little effort and is almost always inexpensive. This is worthwhile and intrinsically satisfying. Some of the papers in this issue are based on more than ten years of data; interpretation of some aspects of these experiments has changed because of data collected in later years.

Lake 226

Lake 226 was divided by a vinyl curtain into two basins and fertilized from 1973 to 1980. This was the first double-basin experiment at the ELA. Initial results of this experiment are summarized in Schindler (1974) and Schindler and Fee (1974). Fertilization of each basin stopped after 1980, and the recovery of the lake was studied from 1981 to 1983. Although lower trophic levels quickly returned to pre-fertilization conditions when additions ceased (Findlay and Kasian 1987; Shearer et al. 1987b), the lake whitefish (*Coregonus clupeaformis*) population responded less rapidly (Mills and Chalanchuk 1987b). Effects of fertilization on lake whitefish may persist for many years.

Experiments in double-basin lakes are more easily confounded than those conducted in single-basin lakes. The greatest problem is the effectiveness of the vinyl curtain that separates the two basins. The curtain in Lake 226 was originally intended to separate the two basins for 3–4 yr. Instead, we maintained the curtain for 11 yr. Leakage between basins occurred more frequently during the final years of this experiment, but had remarkably few effects on results for the fertilization experiment (see papers by Findlay and Kasian 1987; Shearer et al. 1987b; and Mills and Chalanchuk 1987), but had greater impact for an isotope experiment (Hesslein 1987).

Lake 223

Lake 223 was the first whole-lake acidification experiment conducted at the ELA. From 1976 to 1981, the epilimnetic pH was gradually lowered from 6.5 to 5.1, and then it was held at 5.0 to 5.1 from 1981 to 1983. France (1987a, 1987b), Kettle et al. (1987), Shearer et al. (1987a), and Mills et al. (1987) describe some changes in lake biota that occurred from 1976 to 1983. The crayfish papers of France (1987a, 1987b) complement the detailed population studies of I. J. Davies that will appear in a subsequent issue of this journal (I. J. Davies, ELA staff, personal communication). Other chemical and biological changes in Lake 223 are summarized in the recent papers by Schindler et al. (1985), Schindler et al. (1986), and Cook et al. (1986). In addition, many of the studies conducted on Lake 223 benefited from expertise gained during the initial eutrophication program at the ELA. For example, primary production studies (Shearer et al. 1987a) benefited from earlier methods development by Fee (1973, 1980). Fish population studies (Mills et al. 1987) benefited from methods developed for the Lake 226 study by Arnason and Mills (1981, 1987), Welch and Mills (1981), and Mills (1985).

Other Experiments

Since the start of the Lake 223 experiment, ELA acidification research has expanded to other studies. Results of two additional whole-lake acidification experiments in Lakes 114 and 302, a bog acidification experiment, continuous culture studies of acid and cadmium toxicity to zooplankton, and detailed analyses of long-term atmospheric deposition of nutrients and ions at the ELA are reported in this supplement. Schindler and Turner (1982) give initial results for Lake 114 and Anderson and Schiff (1987), Lawrence and Holoka (1987), Leino et al. (1987), and Turner et al. (1987) present further results for Lake 114 and initial results for Lake 302. The acidification program at the ELA expanded in 1980 to include an experimental wetland acidification and Bayley et al. (1987) give results of simulated acid precipitation on *Sphagnum* sp. and water chemistry. Lawrence and Holoka (1987) present results for zooplankton exposed to low concentrations of cadmium and different levels of acidity in continuous culture vessels. Linsey et al. (1987) present 12 yr of data that show hydrogen ion concentration in ELA precipitation increased dramatically in 1982.

This supplement also contains a number of limnocorral experiments. Anderson et al. (1987a) show that rates of trace metal transport obtained using radiotracers are applicable to stable metal behavior. Schiff and Anderson (1987) show that flocculent, organic-rich sediments (Lake 114) have higher buffering capacity than sandy sediments (Lake 302) and that rates of nitrate and sulfate consumption differ in lake sediments. Anderson and Schiff (1987) then discuss probable fates of reduced sulfur in lake sediments. Tube studies have become an important technique for examining geochemical and microbiological hypotheses at the ELA.

Radiotracers remain an important component of many ELA experiments. Following the previous study of metal radiotracers in an oligotrophic lake (Hesslein et al. 1980), Hesslein (1987) reports results for a similar study in the experimentally fertilized Lake 226. Anderson et al. (1987b) took advantage of a unique opportunity to utilize isotopes added to ELA lakes during previous experiments as sediment markers to examine isotopic mobility and to detect coring artifacts. Although previous ELA studies often utilized additions of small quantities of isotopes, Brunskill and Wilkinson (1987) examined natural sources of radioisotopes as a basis for estimating annual supply to unmanipulated Lake 239.

This supplement contains methods papers relevant to ongoing or future whole-lake experiments at the ELA. Arnason and Mills (1987) present further analyses of violations of assumptions on estimates obtained from mark-recapture experiments. Graham and Turner (1987) examined the effects of acidity on respiration of periphyton communities and implications of this for gross photosynthesis. Because aluminum toxicity is often associated with lake acidification, Playle (1987) evaluated the potential of using ^{26}Al as a toxicological tracer in studies of aluminum accumulation in fish. To aid evaluation of stresses on zooplankton in ELA whole-lake experiments, Lawrence et al. (1987) developed a method for estimating dry weight of zooplankton using geometric approximations of their shapes.

Synthesis

Schindler (1987) synthesizes 18 yr of experience at the ELA as a basis for generalizing responses of ecosystems to anthropogenic environmental stress. He highlights organisms which are useful as indicators of subsequent changes in aquatic ecosystems. In general, measurements of ecosystem function such as primary production, nutrient cycling, and respiration have not been good indicators of the early phases of ecosystem stress, but disappearances of some short-lived species that reproduce rapidly are sensitive indicators of stress in ELA lakes.

While the emphasis during the ELA program has been on whole-lake manipulations, ELA staff have become increasingly aware that long-term data sets for undisturbed, reference lakes are extremely important to distinguish both subtle and catastrophic impacts of stressors from natural variation in aquatic systems. Schindler (1987) discusses the importance of detailed, prolonged monitoring in the ELA context, and discusses possible alternatives when this is not possible.

The Future

Almost two-thirds of the manuscripts in this supplement are directly or indirectly related to ongoing acidification research at the ELA. We are presently studying the recovery of Lake 223 from pH 5.1 to original values (approximately pH 6.5) and are continuing the Lake 302 and the wetland acidifications. Based on small-scale cadmium experiments conducted at the ELA reported here (Lawrence and Holoka 1987) and previously (Marshall and Mellinger 1980), we will start a whole-lake cadmium experiment in 1987. We expect to expand to other atmospheric pollutants in subsequent years.

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Detecting Ecosystem Responses to Anthropogenic Stress[1]

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Recent ecological work on aquatic populations, communities, and ecosystems is reviewed for advances which show promise as early indicators of anthropogenic stress in aquatic ecosystems. Work at the Experimental Lakes Area (ELA) in northwestern Ontario indicates that among the earliest of responses to stress are changes in species composition of small, rapidly-reproducing species with wide dispersal powers such as phytoplankton, and the disappearance of sensitive organisms from aquatic communities. Work elsewhere illustrates that the incidence of morphological abnormalities in benthic invertebrates is also highly sensitive to pollution stress. For several categories of pollutants, this sensitivity of benthic organisms may be due to the greater concentrations of pollutants in sediments than in the water column. Variables reflecting ecosystem functions such as primary production, nutrient cycling, and respiration, were not altered by eutrophication, acidification, or cadmium addition at ELA, and are relatively poor indicators of early stress. Species diversity of phytoplankton was also insensitive to low levels of stress. Mesocosm experiments appear to be fruitful for addressing chemical- or plankton-related problems, but are less useful for addressing community- or ecosystem-level questions. Among population-level approaches, life-table population studies of invertebrates appear to be the most sensitive early indicators of stress on ecosystems.

Relative sensitivities of freshwater and forested terrestrial ecosystems exposed to airborne pollutants are compared. Primary production seems to be reduced at a much earlier stage of air pollution stress in terrestrial ecosystems than in aquatic systems. Soils, like lake sediments, tend to be sinks for pollutants. This may protect the pelagic regions of lakes from influxes of toxins that would occur if watersheds and sediments were unreactive, but cause additional stresses to the fauna and flora of soils and sediments. In extreme cases, high concentrations of toxins may inhibit the replacement of terrestrial producers.

The importance of long-term monitoring in distinguishing natural from anthropogenic stress is discussed. It is suggested that paleoecological techniques be rapidly developed and calibrated with whole-ecosystem experiments to resolve certain inadequacies of past monitoring records.

L'auteur a passé en revue de récentes études écologiques sur les populations, communautés et écosystèmes aquatiques pour y relever les éléments qui promettent à titre d'indicateurs de stress anthropique dans les écosystèmes aquatiques. Des travaux menés dans la Région des lacs expérimentaux au nord-ouest de l'Ontario révèlent que des changements dans la composition spécifique des organismes à reproduction rapide et à capacité de dispersion élevée comme la phytoplancton et la disparition d'organismes vulnérables dans les communautés aquatiques comptent parmi les premières réactions au stress. Des études effectuées dans d'autres régions démontrent que l'incidence de difformités chez les invertébrés benthiques est le résultat de stress entraîné par la pollution. Pour plusieurs catégories de polluants, cette sensibilité des organismes benthiques peut être due à des concentrations de polluants plus grandes dans les sédiments que dans la colonne d'eau. Les variables écologiques comme la production primaire, le cycle de bioéléments et la respiration n'ont pas été modifiées par l'eutrophisation, l'acidification ou l'apport de cadmium dans la région des lacs expérimentaux et constituent donc des indicateurs médiocres de stress initial. Les diverses espèces phytoplanctoniques étaient aussi insensibles à de faibles niveaux de stress. Des expériences sur le mésocosme semblent être utiles pour l'étude des problèmes reliés au plancton et aux produits chimiques, mais ont moins de portée pour ce qui est des questions liées aux communautés ou aux écosystèmes. En ce qui concerne les études de populations, les études des tables de survie d'invertébrés semblent constituer les indicateurs les plus sensibles de stress initial dans les écosystèmes.

L'auteur compare les sensibilités relatives d'écosystèmes aquatiques et terrestres en milieu forestier exposés à des polluants atmosphériques. Dans les écosystèmes terrestres, la production primaire semble baisser à un plus faible niveau de pollution atmosphérique que dans les systèmes aquatiques. Les sols, comme les sédiments lacustres, servent de réservoirs de polluants. En soi, ceci peut protéger les régions pélagiques lacustres de l'entrée de toxines qui aurait lieu si les bassins versants et les sédiments étaient inertes; par contre, ceci entraîne un stress supplémentaire pour la flore et la faune peuplant les sols et les sédiments. Dans des cas extrêmes, des concentrations élevées de toxines peuvent empêcher le remplacement des producteurs terrestres.

L'auteur traite de l'importance de la surveillance à long terme pour distinguer les stress naturels des stress anthropiques. Il propose qu'on mettre rapidement au point des techniques paléoécologiques qui seront étalonnées dans le cadre d'expériences sur un écosytème global afin de pallier à certaines insuffisances des données recueillies par le passé.

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The management of natural ecosystems is a major public concern and a primary mandate for most environmental agencies. Despite the large number and size of such agencies in developed countries, degradation of ecosystems is occurring on a global scale. Many ecologists quickly pass all of the responsibility for such damage to politicians or industrialists. If ecologists accept any responsibility at all, it is merely to admit their reluctance to reduce their complicated calculations and novel discoveries to ecological banalities which every man on the street can comprehend. However, I believe that ecologists and the agencies which fund their work must assume a more direct responsibility. We have not yet developed an ecological science capable either of detecting the stresses[2] imposed upon natural ecosystems by anthropogenic activities or of predicting the long-term effects of such stresses. Often, we cannot "sell" our ecological programs to governments, industries, or the public because we cannot convincingly illustrate our prognoses of doom until ecosystems are severely damaged. This had led the public to become increasingly cynical about the effectiveness of environmental scientists and scientific organizations, e.g. Hall (1986) and Wilk (1985). It is time to develop programs to detect early stages of ecosystem degradation and predict the consequences of a broad range of environmental insults. Some preliminary opinions on the subject follow. These are based on my personal experience during the past 18 years in observing ecosystem responses to both experimentally imposed chemical perturbations and natural stresses in lakes of the Experimental Lakes Area (ELA), northwestern Ontario and on discussions with community and ecosystem ecologists who have similar concerns.

Past Management Approaches

Our basis for managing pollutants in whole ecosystems has consisted almost entirely of three approaches, none of which is well-founded in ecosystem science: (1) short-term bioassays on single species, (2) large, complicated deterministic models, and (3) short-term "impact statement" studies.

Our literature contains few examples of ecosystem-level evaluations. In a review of the ecological effects of toxic chemicals for the U.S. Environmental Protection Agency (EPA), Van Voris and Arthur (Ecosystem Research Center, Cornell University, Ithaca, NY, unpubl. MS) evaluated available information on 204 chemical compounds, by reviewing 162 000 papers, computer printouts, abstracts, and citations. To supplement the search, 250 phone calls were made to state, federal and EPA offices in all 50 states to locate unpublished data. In this search, only 1 270 articles (<1%) dealt with substances which were not on the U.S. EPA's list of priority pollutants. The few field studies were usually only extrapolations or confirmations of laboratory studies made under field conditions. None were done at the community or ecosystem level. The EPA has been concerned about the consequences of regulating the pollution of ecosystems based on results of such short-term, small-scale studies. The EPA-funded Ecosystems Research Center at Cornell University was recently formed to advise EPA on how to correct the situation. The state of "Ecotoxicology" was recently reviewed by the Center (Levin et al. 1984) and by the NRCC (1985) in Canada.

Short-Term Bioassays

Because of their small size and short duration, short-term bioassays do not properly assess the role of species interactions (predation and competition), immigration or emigration, or interrelationships between organisms and biogeochemical cycles. Often, the species used are those which can be conveniently kept in the laboratory, rather than the most sensitive or critical species in natural ecosystems. For example, one or more of these shortcomings caused scientists using such experiments to misinterpret some of the key factors in regulating eutrophication (Schindler 1971, 1977) and to underestimate the damage caused by lake acidification (Schindler et al. 1985). As discussed later, recent comparisons have shown such methods to be much less sensitive than simple population approaches. While laboratory bioassays may be useful as initial screening mechanisms, more comprehensive studies must form our basis for ecosystem management.

Deterministic Models

I believe that deterministic models, based on numerous coefficients which are either picked from the literature or measured in single ecosystems have little to contribute to ecosystem management. My experience has been that such models have usually confused, rather than helped to solve, key management issues. Two examples illustrate this. In the mid 1970's, considerable concern was raised within the International Joint Commission about the results of prominent deterministic models, which predicted that Lake Ontario would never recover from eutrophication despite expensive phosphorus control measures. A review by the IJC Expert Committee on Ecology and Geochemistry, which I chaired, pointed out that model results were inconsistent with case-history studies of other lakes, and urged that the phosphorus control measures not be repealed. Within a few years, there were clear signs of recovery in the lake. Of course, shortly thereafter "improved" deterministic models were predicting recovery too!

More recently, the ILWAS model (Gherini et al. 1985) was developed to predict the course of acidification in lakes. Inputs to the model were many, and based on some very fine empirical science. However, the model was able to simulate output chemistry for three Adirondack lakes while ignoring in-lake chemical processes. Shortly thereafter, empirical work elsewhere showed the widespread importance of in-lake processes. This example illustrates how a complex model can "perform" well even when major forcing functions are ignored. Since that time, in situ processes have been incorporated in the ILWAS model.

Even if such models worked, the enormous data base required to use them precludes their use as a widespread management tool. As a result, the models which have proven effective in management of aquatic ecosystems are usually simple and directly grounded in empirical science — for example Vollenweider's (1968, 1976) for eutrophication and Schnoor and Stumm's (1985) or Christophersen and Seip's (1983) for acidification.

One problem with any model is the difficulty in predicting the replacement of established species by ones which have either been too rare to detect or have invaded from elsewhere, and the effects that such invasions may have on ecosystems. Invasions are often most successful in ecosystems which have already been impoverished by anthropogenic stress (Elton 1958, 1966). Such invasions have been responsible for some of our most devastating ecological problems: for example, Dutch elm disease, chestnut blight, introduction of carp to North America, lamprey in the St. Lawrence Great Lakes.

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[2] I use stress in the general sense to describe both detrimental stimuli and responses, as defined in the dictionary, or discussed by Odum (1985).

Environmental Impact Assessment

Pointed critiques of environmental impact assessments were made by Schindler (1976), Rosenberg et al. (1981), Hecky et al. (1984), and Larkin (1984). All stressed the lack of long-term monitoring in the post-impact period to assess the accuracy of the predictions which had been made during pre-impact studies. Hecky et al. (1984) further emphasized the need for long-term pre-impact monitoring to establish the degree of natural variation in ecosystems. They cautioned that impact assessments were seldom designed to be extrapolable to other ecosystems, and should not be used as a substitute for ecological research in guiding ecosystem management policies.

In summary, while lip service is paid by ecologists and environmental managers to concerns over ecosystem-level perturbations, we have neither knowledge of the ecosystem-level effects of man's activities, nor of where and when such effects can first be detected. Fortunately, pertinent population-, community- and ecosystem-level ecological work has been done for purely scientific, if not for regulatory purposes. While not addressing the problem of protecting ecosystems per se, these works contain a number of clues as to how we might improve our detection of man-induced ecological stresses in the future. In the remainder of this paper, I will describe insightful approaches that seem able to detect early signs of perturbation in ecosystems. I shall confine myself largely to aquatic examples, with which I am most familiar. More ecosystem-level work has also been done on aquatic systems (for example, Majer 1983; Beanlands and Duinker 1983, 1984; Eaton et al. 1986). A few terrestrial studies which I considered to be particularly illuminating are also discussed.

Lessons from Population Ecology

Until very recently, the life-table methods which have long been used by population ecologists to detect stresses on animal populations were ignored by regulatory agencies. These are still not used in any formal regulatory context, even though they have consistently shown effects of pollutants at much lower concentrations than LC-50 approaches. Following Marshall's (1962) work on effects of gamma radiation on *Daphnia*, there has been a revived interest in life-table approaches, both in general theory (e.g. Caswell 1980; Hubbell and Werner 1979) and in applied evaluation of aquatic pollutants (Marshall 1978; Daniels and Allan 1981; Allan and Daniels 1982; Gentile et al. 1982; Walton et al. 1982). Where comparisons were done, these population approaches were far more sensitive than LC-50 tests (Fig. 1). Unfortunately, all examples to date are for organisms with short life-cycles, and there may be practical temporal constraints to applying such methods to long-lived organisms.

More recently, H. Caswell of the Woods Hole Oceanographic Institute has been developing mathematical expressions of perturbation theory based on life-table approaches, which take into account the probability that a given stress will cause life-table parameters to vary outside the normal range. Once verified, these offer promise as powerful tests of population perturbation, also indicating the most sensitive parts of the life-cycle.

The use of population studies to provide the basis for water quality regulation is exemplified by Muhlbaier and Tisue (1981), who compared both population-level and LC-50 stress thresholds for zooplankton to projected increases in cadmium concentration in Lake Michigan (Fig. 2). Again, the long-term toxicity thresholds for populations were much lower than those suggested by LC-50's. This study shows that zooplankton populations in the lake may be affected decades sooner than LC-50's indicate if present rates of increase in cadmium continue. One wonders how frequently our water quality standards are too lenient, because our tests are too insensitive.

In summary, population ecologists clearly have established sensitive methods which could be used to upgrade the protocols for ecosystem protection.

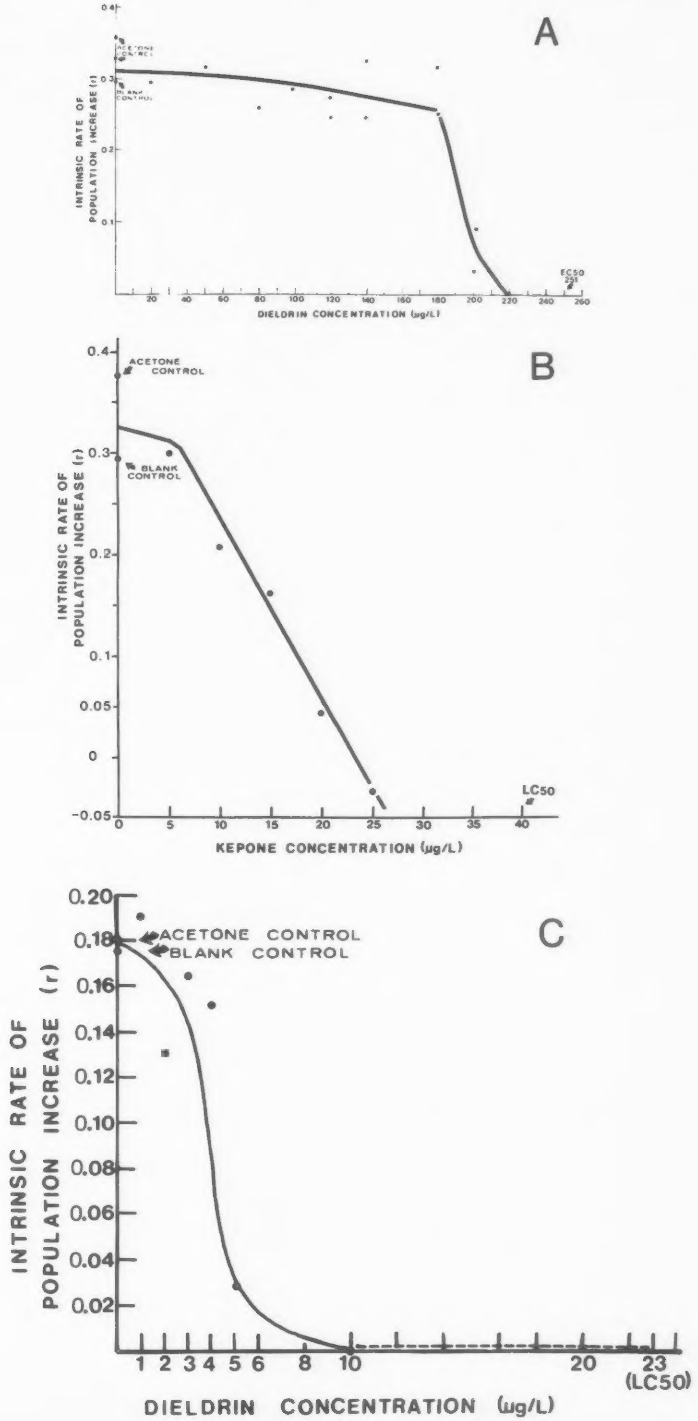
Community Studies

One direct application of community ecology to assessment of ecosystem perturbation has been the analysis of diatom communities by Ruth Patrick and her co-workers (Patrick 1949; Patrick et al. 1954). By analyzing the species assemblages of diatoms which colonize glass slides, Patrick's group has detected low-level effects of perturbations on lotic systems, furnishing information useful for regulating inputs of several types of pollutants. At the ELA, changes in the species composition of phytoplankton are usually among the most sensitive indicators of ecosystem stress, although diversity indices do not appear to fully reflect this sensitivity, as discussed later.

The removal of predators frequently disrupts community structure (Brooks and Dodson 1965; Paine 1971, 1974; Levin et al. 1984; Nero and Sprules 1986; Hessen and Nilssen 1986), so that any perturbation affecting top predators can be expected to have ramifications for the entire ecosystem.

One of my first experiences as an ecologist was to observe the spectacular effect that the elimination of a predator can have on an aquatic ecosystem. From 1963 to 1965, I studied Severson Lake, a small, hypereutrophic lake in western Minnesota. Cyanophyte blooms were so enormous that one could see the path left by a boat in the algae for several minutes after passing. Secchi depths were only a few tenths of a metre, and phytoplankton chlorophyll was several hundred $\mu g \cdot L^{-1}$ in summer. Macrophytes were few and limited to water less than 0.5 m deep. Two full years and two summers of study, plus several years of both intensive and occasional collections by G. W. Comita between 1954 and 1964, revealed a relatively stable composition of zooplankton species, all forms being less

FIG. 1. Examples of the use of intrinsic rate of increase (r) and carrying capacity (K) as measures of the effects of various factors on population dynamics. A. Effect of dieldrin concentration on r for *D. pulex*. The 48-h EC50 is shown for comparison (Allan and Daniels 1982). B. Effects of kepone on r for the copepod *Eurytemora affinis*. The 48-h LC50 is shown for comparison (Allan and Daniels 1982). C. Effects of dieldrin on r for the copepod *Eurytemora affinis*. The 48-h LC50 (23 $\mu g/L$) is shown for comparison (Daniels and Allan 1981).


A
0.4
0.3
0.2
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INTRINSIC RATE OF POPULATION INCREASE (r)
20
40
60
80
100
120
140
160
180
200
220
240
260
EC50 251
DIELDRIN CONCENTRATION (µg/L)
B
0.4
0.3
0.2
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ACETONE CONTROL
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INTRINSIC RATE OF POPULATION INCREASE (r)
0
5
10
15
20
25
30
35
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LC50
KEPONE CONCENTRATION (µg/L)
C
0.20
0.18
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INTRINSIC RATE OF POPULATION INCREASE (r)
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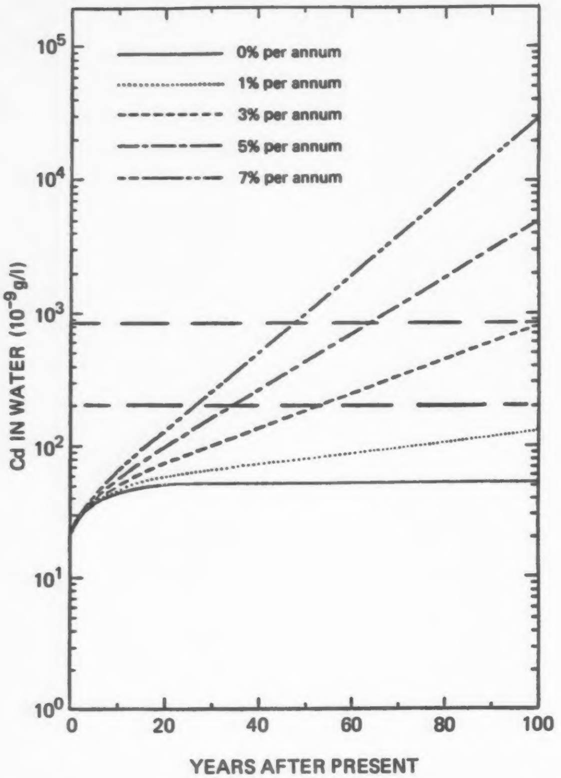

FIG. 2. Projected cadmium concentrations in Lake Michigan for various rates of increase in the annual input of Cd. The lower horizontal dashed line represents the concentration at which toxic effects have been reported for aquatic organisms based on zooplankton community changes (Marshall and Mellinger 1980). The upper dashed line is the current U.S. EPA standard for waters of Lake Michigan's hardness. The present rate of increase of the input rate for Lake Michigan is 4.6% per annum, which would cause the lake to reach the toxicity threshold for aquatic organisms in about 40 years. The authors indicated that a high proportion of the cadmium influx is from atmospheric deposition. When cadmium in sediment was 10^{-6} g·g^{-1} the concentration in water was 1.5×10^{-8} g·L^{-1} (from Muhlbaier and Tisue 1981).

than 1 mm in overall length (Schindler 1966; Comita 1972; Schindler and Comita 1972).

In the winter of 1964–65, total anoxia under ice eliminated fish from the lake. Species eliminated included the green sunfish, *Lepomis cyanellus*, which preyed heavily on zooplankton. In May 1965 large *Daphnia pulex* first appeared in the lake. This species outcompeted other crustacean zooplankton and kept chlorophyll to a few μg·L^{-1}, largely as chlorophyte blooms. The resulting high water transparency (the Secchi disc visibility exceeded the maximum lake depth of 5 m) allowed macrophytes to invade quickly. By mid July, they dominated the littoral zone to depths of 3.5 m. Thus, the simple elimination of a top predator appeared to cause the lake to become structured as a littoral, rather than a pelagic system.

Manipulating predators has become a very fashionable area of ecological research (see review by Zaret 1980). However, few research groups are attempting to use this characteristic of aquatic communities to manage stressed ecosystems. Shapiro and his colleagues (Shapiro 1980; Lamarra 1975; Shapiro and Wright 1984) have tried "biomanipulation" as a eutrophication-control strategy in mesocosm-scale experiments. Similar strategies have been tried in Europe by Stenson et al. (1978) and Henrikson et al. (1980). However, the large differences in redundancy in different natural communities may render the success of biomanipulation difficult to predict unless we have a more complete understanding of species interactions and possible species replacements at all trophic levels. For example, when acidification or other factors eliminate predatory fishes from lakes, they are often replaced by carnivorous insects (Henrikson et al. 1980; Nilssen 1980). In any case, the harvesting of top predators is common in aquatic ecosystems. It appears to have enormous potential for disrupting the structure of entire food webs. Unfortunately, in North American fisheries science, the dynamics of predator populations have usually been studied without reference to the fishery's supporting food web, and most of the models currently used for fisheries management totally disregard the relation between fish stocks and other ecosystem components.

Similarly, application of pesticides to agricultural crops often affects insect predators for longer periods than the herbivorous pests that were the target organisms. As a result, after an initial reduction in numbers, herbivorous pest populations often recover to densities higher than those before spraying (Pimentel and Edwards 1981).

In summary, top predators appear to merit special attention in ecosystem management. Their position at the top of the pyramid of energy means that they are relatively scarce under natural conditions. Although harvesting top predators is often the topic of primary concern to managers of aquatic ecosystems, the effect of their removal on the rest of the food chain is usually ignored. The study of food-chain interactions involving top predators is seldom amenable to any small-scale or short-term assay, and we must adopt entirely new regulatory approaches to include them. I predict that advances in this area of ecology will resolve much of the unexplained variation in responses of different ecosystems to the same perturbation. The recent generalization of food-chain structure into a community-level "trophic cascade" hypothesis (Carpenter et al. 1985) is an important step.

Ecosystem Studies

Few single ecosystem-level studies of perturbation have incorporated a broad variety of organizational, nutrient-cycling, and energy-flow aspects; thus it is impossible to compare them. The literature is badly confused, with the terms "community" and "ecosystem" frequently used as synonyms, even by eminent ecologists, and experiments in "mesocosms" often confused with those in entire ecosystems. Despite the accepted definition of an ecosystem as a biological community plus its nonliving environment (Odum 1963), the term "ecosystem studies" has usually been used to refer to work exclusively on *functional* parameters (energy flow, nutrient cycling), while studies of food-web structure, and population interrelationships are usually regarded as community ecology. Studies which concentrate solely on ecosystem function are no more "ecosystem-level" than those restricted to the study of species interactions, and I shall restrict my focus to studies which have included both, at least for several biological groups and chemical features. As we have shown repeatedly in experimental lake manipulations, chemical cycles and organisms are intimately coupled to the extent that many of the biological changes that follow chemical perturbations clearly help to mend disrupted biogeochemical cycles (Schindler 1985). For example, increasing the input of phosphorus to aquatic ecosystems usually causes dominance of algal communities by nitrogen-fixing bluegreens which, in turn, increase nitrogen inputs (Schindler 1977; Smith 1983; Smith 1984) and cause changes in food resources for zooplankton (Porter 1973). Atmospheric carbon inputs to lakes are also enhanced by increased phosphorus. Typical algal assemblages are replaced by species adapted to low concentrations of CO_2 (Schindler et al. 1972; Schindler and Fee 1973). Both addition of sulfate and eutrophication cause increased activity of sulfate-reducing bacteria in Precambrian Shield lakes, possibly disrupting the iron cycle through precipitation of iron sulfides and enhancing decomposition by sulfate reducers (Schindler et al. 1980a; Kelly et al. 1982; Cook and Schindler 1983). Indeed, the alkalinity of lakes, once thought to be controlled strictly by geochemical weathering, is now known to have a large biological component (Schindler et al. 1986; Schindler 1986). After 18 years of manipulating whole ecosystems, I find that changes in ecosystem function, such as production, decomposition, or nutrient cycling, cannot be properly interpreted without analogous information on the organization and structure of the biotic communities which perform the functions.

Mesocosm and Microcosm Studies

Given the importance often attached to predators in determining the biological composition of ecosystems, as discussed above, it is difficult to see how micro- or mesocosms can provide a reliable basis for ecosystem assessment where either predators or large herbivores are of primary concern. For example, the density of lake trout in Canadian Shield lakes is often about 10 animals per hectare. How large a mesocosm would be necessary to test interactions between elk and grizzly bear, moose and timber wolves, or whales and their Soviet and Japanese predators? Even the very large aquatic enclosures used for experiments lasting more than a few weeks develop abnormally high populations of periphyton (Levine 1983) and artificially prevent replenishment of nutrients from lake watersheds (Lund 1975). The rates of water and nutrient exchange between aquatic mesocosms and the lake in which they are deployed are usually ignored, and are often surprisingly significant. This is not to say that micro- and mesocosm studies have not been useful. They do simulate some features of real ecosystems, if experiments are not of long duration. We and others (e.g., Schindler et al. 1971, 1980b; Brunskill et al. 1980; Marshall and Mellinger 1980; Levine 1983; Gächter 1979) have used them frequently to assess the effects of nutrients and toxins on freshwater plankton and other microorganisms, and on chemical processes. Our efforts to include fish in any "ecosystem scale" experimental design have not been successful. Mesocosms have been similarly used for studies of toxins in marine systems, for example by the MERL project (Pilson and Nixon 1980; Santschi 1985) and CEPEX (Menzel 1977). However, to label such work "ecosystem-level" ignores the important effect of "trophic cascading", as discussed above. While mesocosms are a useful intermediate step between bioassays and ecosystem experiments, they are too removed from real ecosystems to provide a reliable basis for managing them.

Natural Variation and Detection of Low-Level Perturbations

Most of the well-studied perturbations that have severely disrupted ecosystem or community organization or function have been "sledgehammer blows". Ready examples include the toxic gases, acids, and metals at Sudbury, Ontario (Freedman and Hutchinson 1980); the eruption of Mount St. Helens (Baross et al. 1982; Dahm et al. 1983); clear-cutting and herbicide treatment of whole ecosystems (Likens et al. 1970; Bormann et al. 1974), heavy pesticide applications (Pimentel and Edwards 1981; Eaton et al. 1986), large doses of gamma radiation (Woodwell 1962), and 10-fold increases in fluxes of nutrients and acids to lakes at ELA (Schindler et al. 1973, 1980a, 1985).

Such studies are useful, but it would be risky to rely on them alone to predict responses of natural ecosystems to slowly increasing low-level perturbations. The dilemma we face is simply expressed: how do we identify when critical, measurable variables begin to vary outside the normal range, thereby indicating that the ecosystem is perturbed or stressed?

The fact is that we usually do not know the normal range for any variable, at least for any time period greater than a few years. The few longer-term data sets which have been collected show some remarkable trends. For example, it is now known that marine fishes undergo natural cycles with periods of hundreds, or perhaps thousands of years, possibly explaining at least some of the mysterious declines in species in this century (Soutar and Isaacs 1969; Steele 1985). Similar cycles, of several years duration, have been noted for fur-bearing mammals (e.g., Elton and Nicholson 1942a, b). In Swedish rivers, the rate of pH decrease was so small compared to natural variation in pH that its statistical significance was detectable only after many years of measurement (Fig. 3a). The increased average nitrate content of streams at Hubbard Brook would be equally impossible to discern without long-term data (Fig. 3b). Smayda (1984) has shown that at least some of the apparent long-term nutrient increase in Narragansett Bay is due to natural cycles with durations of 5 to 10 years.

Our inability to convincingly distinguish slow unidirectional changes in ecosystems caused by anthropogenic perturbations from natural variation is reflected in our failure to convince regulatory agencies that acid precipitation and other pollutants that are transported for long distances in the atmosphere have serious consequences for natural ecosystems. Likens (1983, 1984, 1985) has emphasized the importance of long-term monitoring studies. How many of us would confidently extrapolate from a 6-year continuing increase in a particular measurement? Most, I think. Yet there are examples from ELA where interpretation of such data would have led to misleading conclusions about long-term ecosystem function.

I shall relate one example in detail, for it illustrates the valuable role that long-term baseline studies can play in deducing stress. Lake 223, experimentally acidified from 1976 to the present, became more transparent for several years as the pH of the lake was decreased, leading both Malley et al. (1982) and Schindler and Turner (1982) to conclude that acidification increased transparency. After all, this conclusion was consistent with the observations on Scandinavian lakes of varying

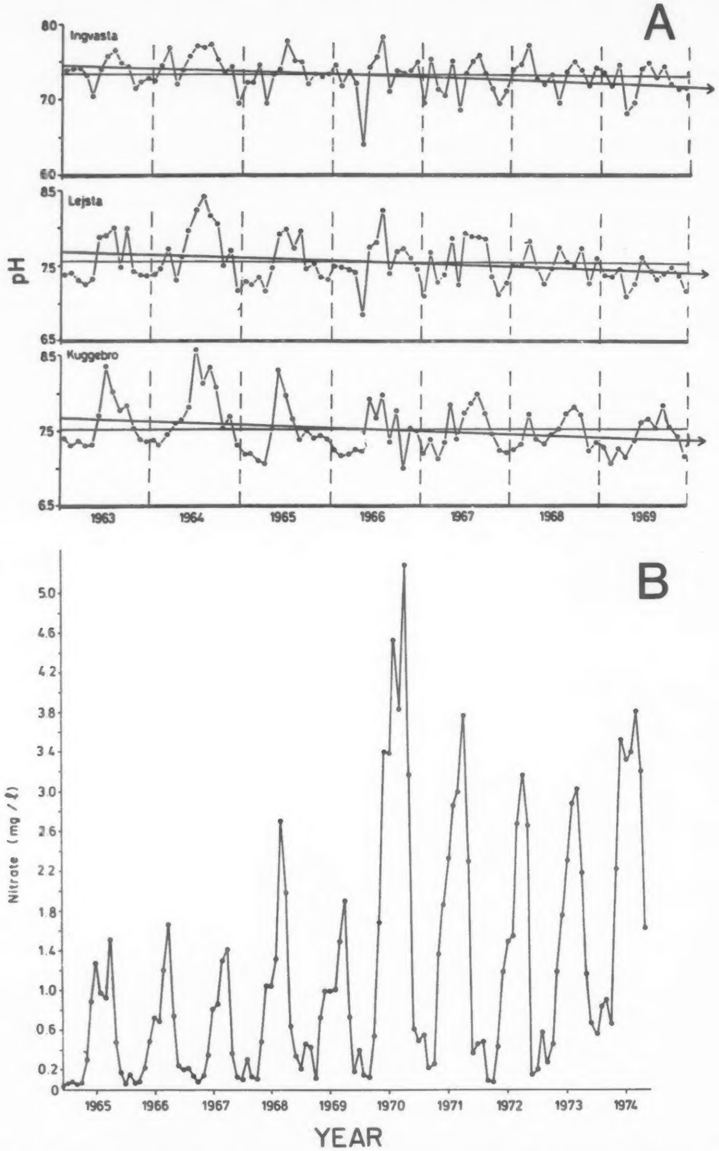

FIG. 3. Examples of cyclic phenomena in aquatic ecosystems. A. Changes in pH values of a Scandinavian river over time. The data points, from three stations in the Savjann River, show the measured seasonal values, and the arrow shows the average pH value (Oden and Ahl 1970). B. Monthly concentrations of nitrate in stream water at watershed 6 in the Hubbard Brook Experimental Forest 1964–74. The time-trend is highly significant ($P < 0.001$) and the correlation coefficient (r) is 0.33 (Likens et al. 1977). For longer-term examples see Steele (1985).

acidity (Grahn and Hultberg 1971) and of studies in eastern Canada (Yan and Miller 1984; NRCC 1981). However, further analysis revealed that transparency had also increased in other ELA lakes during the same period. While the mechanism is not clear, the change appears to have been related to an overall decrease in input of water and several chemical species during several years of below-normal precipitation. Fortunately, more detailed statistical analyses of unmodified reference lakes revealed that our published conclusions about transparency were probably correct (Fig. 4). One wonders how many ecological "responses" have been reported, which are really just natural variation. Investigators seldom have reference ecosystems at their disposal and tend to ignore them if they are available. At ELA, our study of reference (unperturbed) sys-

TABLE 1. Pattern of decline of forest ecosystems experiencing air pollution stress. (from Bormann 1983).

Stage	Level of anthropogenic pollution	Severity of effect on ecosystem	Expected ecological effects
0	insignificant	pristine, no effect	none
1	low level	relatively unaffected	ecosystem serves as a sink for pollutants
2A	levels adverse to sensitive organisms	if continued, possible significant changes in competitive ability of sensitive species	reduced photosynthesis, changes in vulnerability to insect or fungus attacks, deleterious effects on nutrient cycling
2B	increased pollution stress	resistant species replace sensitive ones in dominance	sensitive species lost from the system, mostly due to secondary effects such as fungal or insect attacks, failure in pollination due to deleterious effects on honeybees or other sensitive animals
3A	severe levels of pollution	large-scale changes in original system	large plants, shrubs, and trees of all species perish, toxic concentrations of accumulated pollutants limit many species. Ability of system to regulate biogeochemical cycles severely diminished
3B	very heavy pollution	completely degraded ecosystem	ecosystem collapses

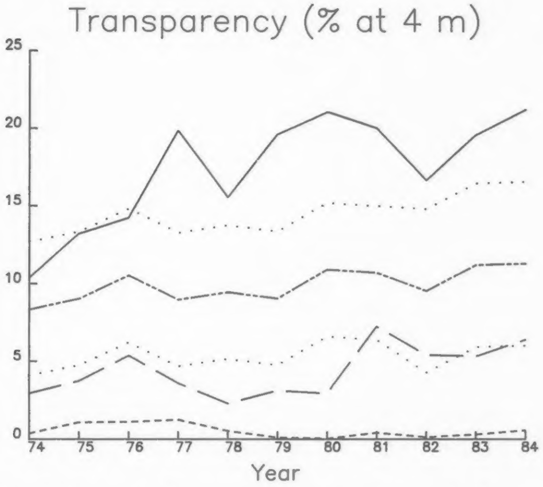

FIG. 4. Average ice-free season transparency in several reference lakes at ELA. Reference lake mean —·—, confidence limits .... and transparency in the eutrophied Lakes 227 - - - and 226 N — — and in the acidified Lake 223 _____ .

tems has been more thorough than most; yet in retrospect, it should have been still more intensive.

For detection of the effects of low-level perturbation on ecosystems, perhaps our most immediate effort should be to perform well-designed, long-term monitoring of sensitive ecosystem variables. A similar opinion is advanced by Likens (1985). It is important to do such monitoring in relatively unperturbed reference areas as well as in perturbed ones to define the normal period and amplitude of natural cycles in key populations, chemicals and other variables. There is some evidence from our ELA studies that even low-level perturbations cause increases in the amplitude of oscillation of sensitive variables, while mean values may not change. For example, addition of phosphorus and nitrogen to Lake 227 caused great oscillations in CO_2 and in phytoplankton biomass. These were damped after several years, while mean population sizes remained similar. Increased oscillations in phytoplankton populations of nutrient-enriched Arctic lakes were also noted by H. E. Welch (Freshwater Institute, Winnipeg, pers. comm.). Unfortunately, in the North-Temperate Zone, no uncontaminated ecosystems remain. Pesticides, trace metals, and anthropogenic sulfates are being deposited even in remote Arctic areas (Herron et al. 1977; Boutron and Delmas 1980). As a result, we cannot be certain that key variables in many of our most widespread and important ecosystems are still unaffected.

Stages of Ecosystem Perturbation

Much of northern North America may be perturbated only to the level which Bormann (1982, 1983) would categorize as Stage 1, in which the ecosystems serve as sinks for pollutants, but without detectable adverse effects (Table 1). However, Bormann and many other forest ecologists believe that forests in much of eastern North America have already deteriorated to Stage 2a and 2b, in which reduced productivity and nutrient cycling, and declines or losses of sensitive species are already occurring. Degradation of sensitive aquatic ecosystems in the East has been known for some time (Likens et al. 1972; Schofield 1976). We will have to evaluate future changes in these systems relative to current conditions, rather than as deviations from natural background. This mistake should not be repeated in other regions, because a few decades hence we could easily find ourselves lacking information on any unmodified ecosystems in the major biomes of the world. As Bormann has pointed out, while there are still many ecosystems where the *effects* of anthropogenic pollution cannot be detected, there are no areas where *pollutants* cannot be detected. The problem of deducing baseline conditions in ecosystems and selecting sensitive variables to monitor is discussed below.

Monitoring to Detect Ecosystem Perturbations

Monitoring programs which will prove useful in the decades ahead must have a number of characteristics. They must be inexpensive enough to survive budget cuts in funding agencies during periods of austerity. They must be simple and verifiable, so that they are little affected by changes in personnel. Finally, they must include measurement which are highly sensitive to changes in ecosystems. Our present programs are far from ideal, and it is important that monitoring be developed as a science in its own right, rather than be the uncritical application of convenient contemporary techniques, which is unfortunately the usual approach to monitoring. At present, there is little incentive for creative scientists to address this serious problem. No scientist would be considered for tenure in a major university department with only monitoring studies on his publication record. The attitude of government departments is no better. Despite intensive research on eutrophication in the early 1970's and the implementation of several nutrient-management schemes, very little monitoring is devoted to the problem today. Acid-rain monitoring programs, many only 3–5 years old, are already falling from favor. As Larkin (1984) has pointed out, our current poor understanding of ecological processes severely limits our ability to design effective monitoring programs. Still, there are a few recent signs of improvement, for example, the approaches by Skalski and McKenzie (1982) and Hinds (1984), and the recent initiation of the Long Term Ecological Research Program by the U.S. National Science Foundation.

One goal of the Experimental Lakes program has been to examine how different ecosystems respond to controlled application of various pollutants. A great difficulty in conducting ecosystem-scale experiments is that it is impossible to replicate treatments exactly, because it is impossible to find lakes with similar morphometries, nutrient recovery, water renewal rates, and food chain structures. Analysis of covariance has proved useful in such situations, for example in evaluating the relationship between nutrient income, water renewal, and a variety of ecosystem responses (Schindler et al. 1978). We are preparing a more general method for detecting changes in ecosystem variables outside the range expected from natural variation.

However, similar statistical dilemmas are faced by managers, for lakes stressed by anthropogenic pollution are not replicates, either. I believe that the secret of dealing with this problem lies in collecting good reference information so that it is possible to detect whether key variables in perturbed lakes being to deviate significantly from the range expected for unperturbed systems. Based on our experimental studies and the impressions from the literature which I have discussed above, I can venture some insights on potentially sensitive lacustrine variables and on which of these can be easily and accuratelv monitored.

The Sensitivity of Ecosystem Functions to Perturbation

As I pointed out above, the variables which have usually been used to characterize ecosystem-level responses are whole-system functions such as production, decomposition, and nutrient cycling. While eutrophication has accelerated these processes in aquatic ecosystems, I have not seen convincing evidence that any perturbation, whether natural or anthropogenic, has been sufficiently extreme to cause long-term decreases in these three variables in an aquatic ecosystem. This is clearly illustrated by studies of production and decomposition in lakes in the blast zone of Mount St. Helens (Dahm et al. 1981, 1983; Baross et al. 1982) and in the production and nutrient cycling of newly-flooded reservoirs in permafrost areas (Hecky and Guildford 1984; Planas and Hecky 1984), in addition to our studies in acidified lakes (Schindler et al. 1985).

Other ecosystem characteristics appear to be sensitive indicators of specific perturbations (for example, loss of alkalinity or increase in aluminum due to acidic precipitation, or increased turbidity due to erosion), but these are not direct indices of ecosystem function.

At ELA, increases in phytoplankton biomass and production accompanied both eutrophication and acidification (Fee 1979; Schindler et al. 1978; Schindler 1980; Schindler et al. 1985), although increases in acidified lakes were largely due to natural variation. Phosphorus, nitrogen, and carbon all increased during eutrophication, either due to direct addition of the elements to the lakes or to secondary reactions with the atmosphere to balance nutrient cycles (Schindler 1976, 1977, 1985). Acidification did not decrease rates of decomposition (Kelly et al. 1984; Schindler et al. 1985) or sulfate reduction and denitrification (Rudd et al. 1986; Schindler et al. 1986). Phosphorus nitrogen cycling are unhindered in acidified lakes (Schindler and Turner 1982), in contrast to common belief. Similar, but tentative, conclusions can be reached from studies of cadmium addition to 150-m^3 mesocosms (Marshall and Mellinger 1980; D. W. Schindler unpublished data). In acid bogs, nitrate removal and sulfate reduction occur at high rates, even at pH values less than 4, evidence of the adaptability of the microbial community (Bayley et al. 1987; Herlihy and Mills 1986).

All of this might logically have been expected, for natural systems contain feedback mechanisms which buffer them against perturbations (Waddington 1957). As a result, monitoring ecosystem functions would be a poor approach to detecting early signs of impending ecosystem damage.

Other Indices of Stressed Aquatic Ecosystems

While ecosystem functions were not diminished, dramatic changes in phytoplankton species occurred due to the above experimental perturbations at ELA. In all of the above-mentioned examples, the list of phytoplankton species after perturbation bore little resemblance to that prior to perturbation. Phytoplankton are small, rapidly reproducing species, with wide powers of dispersal. Up to 300 species are probably present in a single lake, and it is not surprising that an assemblage can be maintained which is capable of performing primary ecosystem functions over a wide range of conditions. The chrysophyceans and diatoms that dominate typical natural lakes at ELA were replaced by chlorophyceans and cyanophyceans in both acidified and eutrophied lakes (Schindler 1977; Findlay 1984; Yan and Miller 1984; Schindler et al. 1985). In acidified lakes, a further succession to acidobiontic diatoms and dinophyceans occurred (Schindler et al. 1985). Zooplankton communities in lakes are also closely linked to characteristics of the particular system. As for phytoplankton, the availability of appropriate species to increase when conditions change is often ensured by high rates of immigration (Patalas 1971). As mentioned, life-table analysis of zoo-

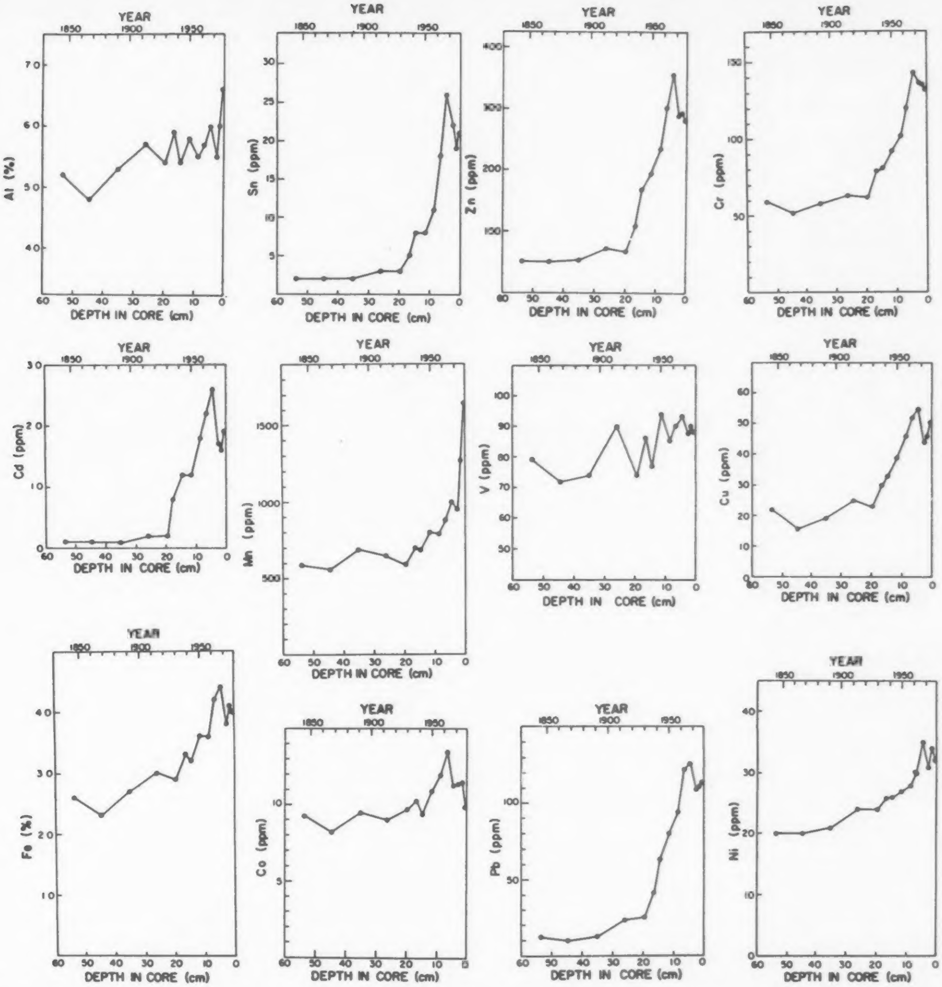

FIG. 5. Metal concentrations as a function of depth and year of deposition in a sediment core from lake Michigan (Goldberg et al. 1981).

plankton has proven to be a very sensitive indicator of several types of chemical pollution, and this approach deserves further study as a possible warning of ecosystem-level perturbations. This has already been done at the mesocosm level for low-level perturbation with cadmium (Marshall and Mellinger 1980; Lawrence and Holoka 1987). Similar studies applied to other invertebrates may prove equally fruitful. It is particularly interesting that unusual, low-level perturbations seem to increase zooplankton death rates, which are compensated by increased birth rates, leaving the "standing crop" of zooplankton under stress relatively unchanged. A far-sighted ecologist should have been able to deduce 20 years ago that this would be so, from the classic predator–prey experiments of Slobodkin (1962).

Comparing the Sensitivities of Forest and Freshwater Ecosystem Functions

There appear to be some major differences in how terrestrial and aquatic ecosystems respond to the complex mixture of air pollutants which occurs in industrialized regions. In contrast to the robustness exhibited by aquatic production as discussed above, terrestrial production appears to be reduced at quite low levels of air pollution (Table 1, Bormann 1983). Ozone and other gaseous pollutants appear to be the major culprits (Hutchinson and Meema 1986; Bormann, pers. comm.). Indeed, plants with buried vegetative organs and other adaptations to minimize exposure to the atmosphere appear to be more resistant to air pollutants (T. C. Hutchinson, University of Toronto, Toronto, Ont., pers. comm.). The movement of toxic gases across the air–water interface is very slow, so that the effect of gaseous pollutants on submerged plants is slight.

Another difference between terrestrial and aquatic communities may lie in the role of soils in plant production. Terrestrial soils and aquatic sediments concentrate most toxicants (Table 2, Fig. 5), including metals, pesticides, and other organic compounds. The major primary producers in most lakes that have been studied (phytoplankton) have little or no contact

TABLE 2. Distribution coefficients $\left(K_d = \frac{\text{concentration in sediment}}{\text{concentration in water}}\right)$ for various substances in freshwater lakes. Values are given to the nearest power of 10.

Substance	K_d	Reference
^{60}Co	10^4	Booth (1976)
^{65}Zn	10^3	Booth (1976)
^{239}Pu	10^5–10^6	Edgington et al. (1976)
^{137}Cs	10^4–10^6	Edgington et al. (1976), Jinks and Wren (1976)
^{90}Sr	10^4	Booth (1976)
Cr	10^4	Booth (1976)
Ni	10^5	Booth (1976)
Pb	$>10^5$	Davis and Galloway (1981)
PCB	10^6	Eisenreich et al. (1981)

with these substrates. In terrestrial ecosystems, the key producer species are rooted directly into soils, where they may be directly affected by contaminants (Hutchinson and Whitby 1977). Rooted macrophytes in contaminated aquatic ecosystems may respond similarly, but critical evidence is lacking.

In contrast to aquatic ecosystems, the dominant producers in forest ecosystems of interest to man are usually large trees and shrubs which reproduce and grow slowly. If killed, it may be years before they are replaced. The ability of the ecosystem to maintain key metabolic functions such as photosynthesis, may therefore be less in forests than in planktonic systems. For example, while long-term acidification has not decreased photosynthesis in lakes (Dillon et al. 1979; Schindler et al. 1985), airborne pollutants appear to have caused such a decline in eastern North American forests, in the Ohio Valley, and in Europe (Table 1, Bormann 1982, 1983; U.S. EPA 1981; Ulrich 1983). Of course, changes to pollution-resistant species must also occur in forest ecosystems, but the replacement rate is much slower (turnover times of years to centuries instead of days), so that decreases in productivity and nutrient cycling are not detectable on the time scale over which ecological measurements are made.

In summary, it appears that nutrient cycling, productivity, and decomposition are not sensitive indicators of stressed aquatic ecosystems. If these attributes do diminish, it is not until an ecosystem is severely degraded. For example, while indicators of ecosystem function such as phytoplankton productivity, nutrient cycling, and decomposition in Lake 223 were unaffected by acidification to a pH of 5.0, recruitment of all fishes had failed by this pH, and a number of key organisms had been eliminated from the food chain (Schindler et al. 1985). Shifts in phytoplankton species assemblages, although taxonomically complex, appear to be far more sensitive indicators of perturbation than measurements of ecosystem function (Schindler et al. 1985). In forest ecosystems, productivity might be a very sensitive stress indicator, but it is very difficult to measure, limiting its diagnostic value. Species disappearances, or shifts to pollution-sensitive species, may again be more useful indicators of stress, but they are more likely to occur first among small organisms, with rapid turnover rates, such as the biota in the litter layer (Whitby and Hutchinson 1974). However, the taxonomic complexity of such groups has limited their use in an early warning context.

Paleoecological and Benthic Studies

As shown in Table 2, aquatic sediments may accumulate pollutants. Thus, the sediments also provide our only long-term historical record of pollutant inputs into aquatic systems. Accurate paleoecological interpretation of these records of pollution, and of the accompanying changes in organisms, will be very important in the next few decades, because even if adequate monitoring is begun soon (an unlikely occurrence), it will be some time before information is sufficient to distinguish between effects of slowly increasing stresses and natural variation. Interpretation of sedimentary records would allow much useful information to be extracted from currently stressed ecosystems, where there are no direct "pre-impact" observations.

Because many pollutants are more concentrated in sediments than in the water column, organisms living in sediments must be exposed to much higher concentrations of pollutants than pelagic or planktonic forms. It is, then, not surprising that some of the organisms considered as sensitive paleoecological indicators of stress live in sediments and leave well-preserved fossils (Frey 1964; Warwick 1980; Wiederholm 1984).

Many benthic organisms are among the most sensitive of higher aquatic species, even to pollutants such as acids, which are not concentrated in sediments. For example, *Mysis relicta* was the first species to be eradicated by acidification of Lake 223 (Nero and Schindler 1983). Other benthic crustaceans, such as *Gammarus* (Økland 1980), *Pontoporeia* (R. W. Nero, Univ. of Toronto, Toronto, Ont.), and *Hyalella* (L. Grapentine, Freshwater Institute, Winnipeg, Man. and M. Stephenson, Guelph Univ., Guelph, Ont., pers. comm.), as well as many species of molluscs (Økland and Økland 1980), seem to be adversely affected during early stages of acidification. Crayfish are only slightly less sensitive (Malley 1980; France 1982; Schindler et al. 1985). Among aquatic insects, the Ephemeroptera seem to include many acid-sensitive species (Wiederholm 1984). The appearance of Zygnemales is often an early botanical indicator of acidification (Stokes 1984), and changes in chironomid communities have long been used as indicators of eutrophication (Thienemann 1925; Lundbeck 1927; Brundin 1956; Saether 1975; Warwick 1980).

Some of the above statements need qualification. *Mysis*, *Pontoporeia*, crayfish, mayflies, and some molluscs are useful as indicators of ecosystem damage only if we know whether they have occurred historically in the ecosystem of concern. Also, detecting changes in species of phytoplankton and emerging insects requires highly specialized taxonomic skills. Many attempts have been made to replace mundane descriptive taxonomy with mathematical indices of diversity or similarity, in the hope that the latter might be reliable early indicators of stress in aquatic ecosystems. However, our observations indicate that detectable decreases in the phytoplankton diversity of acidified lakes occur later than replacement of sensitive original species. Likewise, phytoplankton diversity in experimentally-eutrophied lakes decreases only during summer cyanophycean blooms (Fig. 6). Gray (1979) showed that the diversity of marine benthic communities was not decreased by exposure to sewage, even though species composition was changed. Of course, diversity indices must be based on reliable taxonomy, and the taxonomy of many aquatic groups is

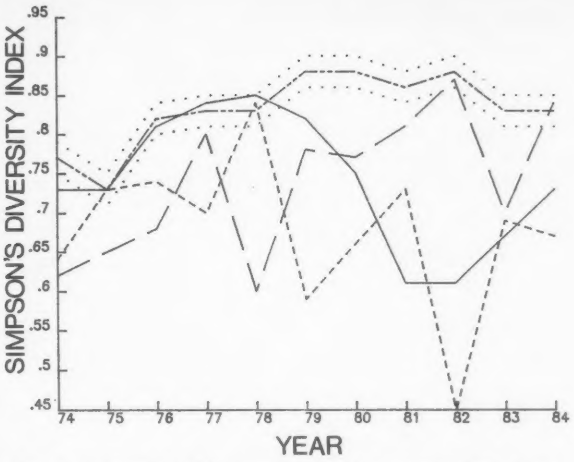

FIG. 6. Simpson's (1949) diversity index for phytoplankton in Lake 223, which was acidified in 1976–83, Lake 227 which was eutrophied in 1969–83; and Lake 239, which had half the drainage basin burned in 1974, and the entire drainage area burned in 1980. Symbols are the same as in Figure 4. Note that the only extreme decreases in diversity were in midsummer in Lake 227 and 223. In the latter case, significant decreases in diversity occurred only in 1981–83, when the pH of the lake was 5.1. (Data supplied by D. Findlay). The apparent increase in diversity in reference lakes is due to improved taxonomy, rather than true increases in diversity.

incomplete, though rapidly improving through the application of modern techniques such as electron microscopy and enzymatic methods (e.g., Cronberg 1980; Grassle and Grassle 1977). Concerted efforts during the past several years have also improved our taxonomic knowledge of freshwater zoobenthos (e.g., Merritt and Cummins 1984; Wiederholm 1983).

The Response and Recovery of Ecosystems under Natural Conditions and Anthropogenic Perturbations

Certain natural phenomena are also devastating in their effects on ecosystems. A striking example is the windthrow and burning of boreal forst, clearly 3b effects on Bormann's scale (Table 1), except that after the ecosystem collapses, it begins an orderly and predictable recovery. This is possible because thousands of years of such events, at intervals of 50–100 yr, have left a biotic community with species adapted to fire and windthrow. Mechanisms include heat-resistant seeds (jackpine), reservoirs of seeds buried in the soil (pin cherry), seeds carried long distances by wind or birds (poplar and fireweed), or resprouting from roots (poplar, alder, and birch). As a result, after burning, the boreal forest ecosystem rapidly recovers control of its nutrient cycles (Schindler et al. 1980c; Bayley and Schindler 1986). Transpiration, and presumably productivity, quickly return to at least pre-fire values (Schindler et al. 1980c).

There are conflicting opinions about the significance of adaptation to fire in other ecosystems. For example, in humid tropical forests of the Amazon, large natural fires are rare. Vogl (1977) hypothesized that the plant communities of such ecosystems are not adapted to fire and would contain few pioneer species. Sites cleared and burned by humans would be

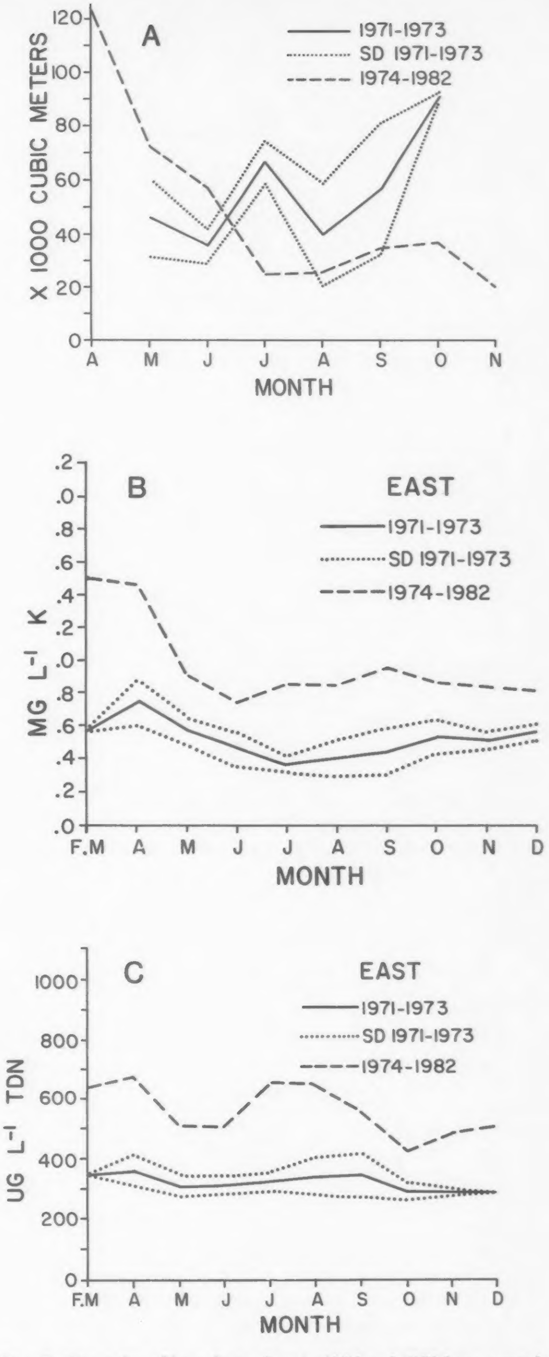

FIG. 7. Examples of how forest fires in 1974 and 1980 have caused long-term disruptions in the water and nutrient losses from a forested watershed. Shown are monthly mean yields and standard deviations for the pre-fire period (1971–73) and the post-fire (1974–82) period. Values are from the East Subbasin of Lake 239. (A) water losses, (B) losses of potassium, and (C) losses of total dissolved nitrogen (from Bayley and Schindler 1986).

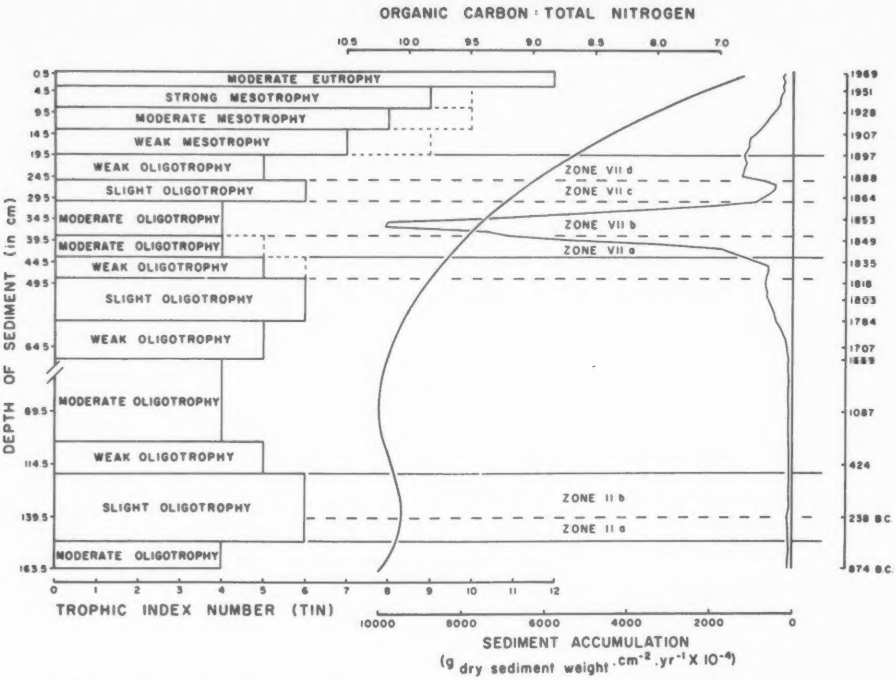

FIG. 8. Influence of mineral sediment accumulation on trophic status measurements, based on productivity indices and chironomid communities (from Warwick 1980).

expected to remain unvegetated for long periods, causing high soil erosion, loss of water and nutrients.

However, in Venezuela, Uhl and Jordan (1984) found that *Cecropia* rapidly recolonized cut-and-burn plots, resulting in leaf area indices close to those in undisturbed plots within 5 years. The genus grows from a seed bank in soils, very similar to pin cherry at temperate latitudes. Swain and Hall (1983) describe another successional analog, *Musanga cecropioides*, from Ghana. Clearly, much more work is needed to define the types and frequencies of disturbance from which ecosystems will recover.

The documented cases of delayed ecosystem recovery seem to involve perturbations that would not have occurred before the advent of the 20th century technology. Erosion and nutrient losses similar to those in the tropics Vogl (1977) were observed at Hubbard Brook, after a watershed was clearcut and revegetation was prevented by herbicide application (Likens et al. 1970). Similarly, forest regrowth near smelters is prevented by accumulation of toxic metals in soils (Freedman and Hutchinson 1980; Hutchinson and Whitby 1977). I have seen one natural example of delayed recovery. Forests at ELA that were burned twice in 6 years have not recovered their full evapotranspiration rate or ability to retain nutrients (Fig. 7). The second burn destroyed young jackpine and poplar, and was so intense near the ground that it burned most of the organic matter (including tree seeds) in the upper several centimeters of soil, and in some areas, shattered a centimetre or more of the bedrock surface. Such double burns are probably too rare in the boreal forest to allow adaptation to occur. They seem to have effects similar to repeated land clearing (Godron 1978). Thus many anthropogenic stresses may resemble infrequent natural ones, in that they occur so rarely that there has been no chance for ecological communities to adapt to them.

It is perhaps even more important to understand the rates at which genetic adaptation can occur without causing elimination of species, because while the elimination of a desirable species is usually unacceptable to naturalists and resource managers, changes in genotype may go unnoticed. At present, we have scanty knowledge of such changes in aquatic ecosystems, although a number have been accidentally discovered. The dipteran *Chironomus riparius* has adapted to pH 3.5 in the ponds of the Smoking Hills of northern Canada, which are believed to have been acidic for millenia, apparently by producing much higher hemoglobin concentration than is normal for the species (Havas 1981; Jernelov et al. 1981). The same species collected from Swedish waters was much less tolerant, with 17 of 20 specimens dead after 20 days in water of pH 3.4. Different strains of brook trout, *Salvelinus fontinalis*, vary in their pH tolerance (Falk and Dunson 1977). Presumably, these differences have evolved since their marine origins 8 000–12 000 years B.P.

Certain adaptations to pollutants have occurred within decades. These include SO_2-resistant geraniums (Taylor and Tingey 1981), moths exhibiting industrial melanism (Kettlewell 1955), metal-tolerant algae and fish (Stokes et al. 1973; Klaverkamp et al. 1984), and DDT-resistant insects (Pimentel and Edwards 1981). Documented cases are too few to assess how widespread such adaptations have been. In many cases, the pollutant-resistant genotype was undoubtedly present in the population at the onset of pollution, so that it simply increased in relative abundance. The question of whether pollutant-resistant genotypes will respond to increased pollution without unacceptable changes in phenotype (at least for species valued by humans) deserves exploration. We must also know whether the favoring of pollutant-resistant genotypes renders species less fit in other ways which are critical to their success.

We would, of course, be less concerned about the elimination of species from a given ecosystem if the species returned quickly once the stress was removed. In this respect, species with cosmopolitan distributions and rapid dispersal abilities have an advantage. Chironomids and phytoplankton are excellent examples. On the other hand, organisms with limited ranges or poor dispersal powers must be of more concern. There will be no return of the passenger pigeon. Poor dispersal powers may also prevent ecosystem recovery. *Mysis*, *Lepidurus*, *Pontoporeia*, *Salvelinus*, and *Limnocalanus* all have contemporary distribution patterns in lakes geographically bounded by the extent of glaciation several millennia ago, and their return to ecosystems from which they have been eliminated by man seems unlikely, unless they are deliberately restocked. Unfortunately, all five of these genera are moderately to greatly sensitive to acidification caused by acid precipitation, which now occurs over 50% or more of their ranges in North America (Mills and Schindler 1986). Studies of the recovery of ecosystems from pollutants are relatively few, and our ability to predict the recovery of different species or communities is poor.

Are There "Quick and Dirty" Solutions?

Even if well-designed monitoring programs were magically emplaced tomorrow, it would be years before we could confidently distinguish between natural variation and low-level effects of perturbations on ecosystems. The popularity of laboratory toxicity tests, impact assessments, and deterministic modelling is due at least in part to the relative speed and ease of obtaining estimates. Yet there are two relatively short-term approaches that are potentially more reliable indicators of ecosystem damage: paleoecology and experimental ecosystem perturbation.

Lake sediments, low temperature, and low oxygen provide excellent storage for ecological material. In ideal locations, sedimentary deposits are stacked so precisely that long-term biological sequences can be reconstructed to within a month or two. Fossils from the lake and surrounding terrestrial ecosystems are preserved concurrently, so that it is possible to deduce changes in both terrestrial (for example, Swain 1973) and aquatic communities (Fig. 8).

Unfortunately, paleoecological methods are also in the early stages of development, particularly for recently deposited sediments, and we cannot always be certain that our interpretation is correct. For example, for many years fossil chironomid assemblages were used as indicators of eutrophication. Increased inputs of silt to the Bay of Quinte, Ontario, from terrestrial watersheds cleared in the 18th and 19th century caused the same change in chironomid fossils as decreased nutrient input (Fig. 7). Once siltation decreased, the original assemblage returned, only to be modified again as eutrophication increased in the 20th century. Without extensive research into the history of the Bay of Quinte ecosystem, and a number of supporting paleoecological analyses (e.g., *Ambrosia* as an indicator of land clearing, Pb^{210} dates to gauge siltation rates, nutrient analyses to allow the degree of eutrophication to be measured), Warwick might have reached erroneous conclusions.

In another example, Davidson (1984) studied the diatom assemblage in the sediments of Lake 223. Standard paleoecological techniques applied to deducing past pH's of lakes incorrectly indicated that the lake had become acid in 1964, not 1976. The reason for this discrepancy was the mixing of surface sediments to a depth of a few centimetres, causing mixing of acidobiontic diatoms deposited in the late 1970's and early 1980's with older strata. Standard paleoecological indices also predicted a pre-acidification pH of 0.5 units lower than that measured in Lake 223 prior to acidification. Such errors, if widespread, would cause the changes due to anthropogenically caused acidic precipitation to be underestimated. Paleoecological methods should therefore be calibrated in ecosystems with known pollution histories.

The increased incidence of pollution in the past few decades also seems to have caused abnormal genetic expression in many benthic species. Abnormal chironomids, other aquatic insects and oligochaetes have been found in sediments of sites polluted with a wide variety of substances (Chapman et al. 1982; Petersen and Petersen 1983; Tennessen and Gottfried 1983; Cushman 1984). In some cases, increased deformities appear to occur before there are any other signs of ecosystem stress, offering promise of a potential early warning indicator. As suggested earlier, it is not surprising that benthic communities are among the first to show evidence of pollution stress. As can be seen from the recent dates of the above publications, the scientific development of such techniques is still in its infancy.

Like so many other branches of ecology that rely heavily on individual expertise rather than on high technology, paleoecology has been the province of very few specialists, and its application in interpreting anthropogenic stresses has been sporadic. Only recently have paleoecological methods been developed for assessing recent environmental problems. Frequently, those performing the work are not trained ecosystem ecologists. We must attempt to increase the quantity and improve the quality of paleoecological work. Calibration of methods in ecosystems that have been experimentally modified or monitored for long periods will be important. Good taxonomy is, of course, indispensible. Formal courses in paleoecology in universities are rare, but they might help to stimulate interest in this under-utilized approach to assessing long-term changes in biological systems.

The Importance of Whole-Ecosystem Experiments

Experimental ecosystem perturbations allow us to separate the effects of different pollutants, to assess early effects of perturbations in systems with known background properties, and to assess quantitatively the result of known perturbations on whole ecosystems. Unfortunately, experiments on such a scale are not possible in many types of ecosystems, for other human interests are usually considered to be more important, or the systems have already been affected. The low priority assigned to experimental preserves seems to me to be a terrible mistake. Large-scale experimentation has played a key role in the development of 20th century agriculture, and the allocation of land and funding for experimental agricultural work is widely accepted. In agriculture, it would be unheard of to proceed directly from laboratory-scale results to massive com-

TABLE 3. General responses of aquatic ecosystems and mesocosms in the Experimental Lakes Area to various chemical perturbations.

1. Phytoplankton production and standing crop remain within rather narrow limits regulated largely by the input of phosphorus and the rate of water renewal, regardless of pollutant stress.
2. The rate of decomposition is governed by primary production (cf. 1) and is not affected by pollutants.
3. Nutrient cycling is not disrupted by inputs of toxicants.
4. The relative abundance of species with short life cycles changes to favor forms that can maintain critical ecosystem functions in the early stages of ecosystem stress (cf. 1–3 above). Such opportunistic species assemblages contain numerous valuable indicators of stress and may serve as a valuable early warning of developing problems in lakes or their terrestrial drainages and airsheds. The original species are generally expected to return when stresses are removed.
5. Mathematical diversity indices are less sensitive as early detectors of ecosystem stress than taxonomic changes per se, because species diversity in taxa that are diverse enough or taxonomically well-known enough to study quantitatively remains constant when ecosystems are toxified. In the few instances where disappearing species were not replaced, there was little or no initial redundancy in that ecosystem component.
6. The most vulnerable species in most ecosystems are organisms with short life cycles and poor powers of dispersal. Of particular concern from the standpoint of ecosystem stability will be locations in the food web where redundancy, immigration, or both are low.
7. In most aquatic ecosystems, sensitive indicators of stress include life-table parameters of sensitive, short-lived species and changes in community structure resulting from elimination of keystone predators.
8. Neither short-term acute toxicity studies nor measurements of ecosystem-level processes are sufficiently sensitive to detect the early stages of ecosystem stress.

mercial application, without intermediate testing in experimental plots. Ecosystem-scale experiments have been vital to the development of managing fish ponds (Vinberg and Lyaknovich 1965). There is a desperate need for analogous approaches in managing natural ecosystems, but such work can be done at few sites. The potential power of such approaches, if combined with vigorous long-term monitoring programs, is clearly visible in studies done at Hubbard Brook and at ELA.

Ecosystem Level Mechanisms for Responding to Perturbation

The responses of ELA lakes to deliberate chemical perturbations are listed in Table 3. At present, these are based entirely upon whole-ecosystem additions of nutrients, acids, and radionuclides, plus results of mesocosm experiments with cadmium where comparisons seem appropriate. Of course, future whole-ecosystem studies of a diversity of pollutants are necessary to verify the list.

Item 1 in Table 3, the maintenance of phytoplankton production and standing crop under a broad range of pollutant stresses, is well known to apply to most lakes. The management of eutrophication based on the simple principles of phosphorus input and water renewal has perhaps been the greatest success in 20th century ecosystem management. Only 15 years ago, there were dozens of hypotheses on how eutrophication should be controlled. Volumes from the eutrophication symposium in Madison (NAS 1969) and the ASLO symposium 3 years later (Likens 1972) contained hypotheses for eutrophication control based on nitrogen, carbon, trace elements, major ions, and light, as well as phosphorus. The rapid progress in this one area can be traced largely to the penetrating work of R. A. Vollenweider and his colleagues (Vollenweider 1968, 1975, 1976; Janus and Vollenweider 1981; Rast and Lee 1978; Dillon and Rigler 1975; Schindler et al. 1978). This rapid progress in managing eutrophication is enlightening in two respects. Firstly, it illustrates that despite the apparent complexity of ecosystems, a number of simple laws govern their behaviour.

Items 2 and 3 in Table 3, which state that neither decomposition nor nutrient cycling has been disrupted by acid ecosystem perturbations, may surprise many scientists. For example, it is widely accepted that acidification decreases rates of decomposition and nutrient cycling. But detailed scrutiny reveals that studies suggesting such disruption are lacking in some respect. For example, cores of sediments and their resident biota are often taken to the laboratory and forced to assume low pH values by mixing with acid. Reduced oxygen demand occurs as a result. Kelly et al. (1984) showed that such treatment does indeed decrease decomposition, but it is not relevant to real ecosystems. In most acidified lakes, sediments a few centimetres below the mud-water interface retain pH values a unit or more above values in the overlying water. Overall, decomposition in acidified lakes is not reduced by acidification to pH 5 (Kelly et al. 1984). Similarly, only at ELA have measurements of nutrient inputs, outputs, and masses been made before and during acidification. These have revealed no major disruption in nutrient cycling (Kelly et al. 1984; Schindler and Turner 1982; Schindler et al., unpublished data). A recent study of the effect of the insecticide chlorpyrifos on experimental stream channels yielded similar conclusions (Eaton et al. 1985).

It is of some interest to compare our results with Odum's (1985) list of trends expected in stressed ecosystems. None of the trends in energetics or nutrient cycling proposed by Odum were observed in our experimental lakes, or in Eaton's streams. In community structure, both eutrophication and acidification tended to cause the average size of organisms to become larger rather than smaller. For example, the natural assemblages of small chrysophyceans were replaced by filamentous bluegreens during eutrophication and large dinoflagellates during acidification. The cessation of fish reproduction during acidification (Schindler et al. 1985), and the increased growth rates of fish during eutrophication (Mills 1985) should have a similar effect. There is some suggestion that food chains shorten, as Odum predicts, and his conclusions about species diversity and dominance appear to be correct, although as I mentioned earlier, individual species and life table analyses appear to be still more sensitive.

Under the category "general system level trends", we seem to have seen the increase in parasitism which Odum predicts — for example, the increase in *Thelohania* and fungal infections on crayfish (France and Graham 1985). The fact that overall lake respiration has not decreased suggests that resource utilization is unimpaired, although we may not value the organisms doing the utilizing as highly as the original species assemblage. Item 18 on Odum's list, the higher robustness of functional than of structural properties, is in fact based on the conclusions of our earlier work, so that agreement is perfect.

Altogether, it appears that we are still far from having a comprehensive view of the ecosystem "stress syndrome", although the improvements documented by Odum over a period of several years indicate that progress is being made. In particular, more aquatic examples are needed. With the exception of our work, Odum had only microcosm and mesocosm experiments to consider at the time of his review.

If my observations and Eaton et al.'s are generally valid, some dramatic changes are necessary in our approach to detecting early signs of aquatic ecosystem degradation. If such low-level detection is our goal, increased emphasis on studies of population dynamics, food-web organization, and intraspecies adaptation and taxonomy must be incorporated in ecosystem-level studies. This is not a call to return to ecology as it was practised 40 years ago, because experimental methods, designs, and approaches today can be much more efficient: for example, the previously-mentioned application of electron microscopy and electrophoresis in taxonomy; the application of sophisticated computer calculations and mathematical perturbation theories to guide our experiments in population and community ecology; and the use of deliberate experimental ecosystem manipulations to test the validity of hypotheses concerning indicator species and communities, ecosystem function, paleoecological methods, and laboratory or mesocosm studies. There has perhaps never been a more critical, more challenging period in the history of ecology.

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Exchange of Nutrients Between Sediments and Water After 15 Years of Experimental Eutrophication

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Schindler, D. W., R. H. Hesslein, and M. A. Turner. 1987. Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can. J. Fish. Aquat. Sci. 44(Suppl 1): 26–33.

Increases in the concentrations of nitrogen and phosphorus occurred in the water column of Lake 227 during 15 years of fertilization with phosphoric acid and sodium nitrate. The sources of the increases were deduced by comparing the budgets of N and P to the budget for sodium, using a simple model, and from the chemistry of sediment pore water.

Total inventories for sodium since fertilization began confirmed that the element was almost perfectly conservative, as expected. When model results for phosphorus and sodium were calculated, the apparent increase in phosphorus was found to be due entirely to lower water renewal rates during recent years. This agrees with the observation that phosphorus concentrations in sediment pore water did not change between 1975 and 1982. Both phosphorus and sodium in the lake have been in equilibrium with external loading for the past several years.

In contrast, the observed increase in nitrogen could not be accounted for by the decreased water renewal rate, implying that the element was either becoming more efficiently recycled or that there was an increasing, unmeasured external source of the element. An increase in pore water ammonium between 1975 and 1982 was balanced by an increase in the concentration in overlying waters, so that diffusive fluxes did not change. Increasing blooms of nitrogen-fixing bluegreen algae after 1975 indicate that N_2 fixation is the increasing source of nitrogen to the lake. No tendency for the nitrogen:sodium ratio to stabilize has been observed, indicating that the nitrogen budget has still not reached a new steady state.

Il y a eu augmentation des concentrations d'azote et de phosphore dans la colonne d'eau du lac 227 pendant 15 années de fertilisation avec l'aide phosphorique et le nitrate de sodium. On a déduit les sources des augmentations en comparant les bilans de N et de P au bilan concernant le sodium en utilisant un modèle simple et à partir de la chimie de l'eau de porosité des sédiments.

Tous les inventaires faits pour le sodium depuis le début de la fertilisation ont confirmé que l'élément était presque parfaitement conservateur comme prévu. Quand les résultats du modèle pour le phosphore et le sodium ont été calculés, l'augmentation apparente du phosphore s'est avérée être attribuable entièrement aux taux plus faibles du renouvellement de l'eau au cours des dernières années. Cela concorde avec l'observation que les concentrations de phosphore dans l'eau de porosité des sédiments n'ont pas changé entre 1975 et 1982. Le phosphore de même que le sodium dans le lac ont été en équilibre avec l'apport externe au cours des dernières années.

Par contre, l'augmentation observée d'azote ne pouvait pas être expliquée par la diminution du taux de renouvellement de l'eau, ce qui signifie que l'élément s'était recyclé plus efficacement ou qu'il y avait une source extérieure croissante et non mesurée de cet élément. Une augmentation d'ammonium dans l'eau de porosité entre 1975 et 1982 a été équilibrée par une augmentation de la concentration dans les eaux qui sont au-dessus, de sorte que les débits de diffusion n'ont pas changé. Des proliférations accrues d'algues bleu-vert fixatrices d'azote après 1975 montrent que la fixation de N_2 est la source croissante d'azote pour le lac. On n'a observé aucune tendance de stabilisation du rapport azote/sodium, ce qui indique que le bilan de l'azote n'a pas encore atteint un nouvel état de stabilité.

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During the past 15 years, a number of models have been developed and tested which predict steady-state concentrations of nutrients and phytoplankton in lakes from average rates of nutrient input and water renewal (for example, Vollenweider 1968, 1976; Dillon and Rigler 1975; Schindler et al. 1978; Janus and Vollenweider 1981). All of these models apply to lakes at steady state, but we still do not know how long it takes for a lake to equilibrate with a change in nutrient input. At the core of the problem is our poor understanding of the processes which control steady state, particularly the exchange of nutrients between lake water and sediments. Following Mortimer's (1941 – 42) classical work, limnologists have usually assumed that the release of phosphorus from sediments is controlled largely by the oxidation-reduction cycle of iron at the sediment – water interface (see also Golterman et al. 1977). On the other hand, redox reactions are not sufficient to explain sediment – water interactions in Lake 227, a lake which has been eutrophied by fertilization with phosphorus and nitrogen since 1969 (Schindler et al. 1971, 1973, 1977; Schindler 1975). Instead of being

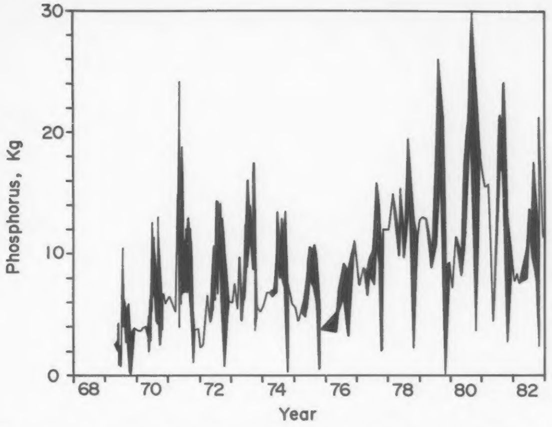

FIG. 1. The mass (kg) of phosphorus in Lake 227, 1968–82. The upper line is the whole-lake mass, the shaded area the mass in the epilimnion. The epilimnion in this and Fig. 2 is defined as the volume above the depth where temperature changes are equal to or greater than 1°C per metre of depth.

directly controlled by the iron cycle, phosphorus is initially taken up by organisms in the water column and surficial sediments (Levine and Schindler 1980). Some of it is excreted as large organic molecules (Schindler and Lean 1974), and the eventual binding appears to be as high molecular weight organic – metal – phosphorus complexes (Jackson and Schindler 1975).

In contrast, ammonium fluxes from the sediments can be a substantial contribution to the nitrogen cycle of Lake 227 (Schindler et al. 1977; Hesslein 1980a and b). In addition, nitrogen fixation occurred in the lake in 1975 and thereafter, when the N:P ratio in input was reduced (Schindler 1977, 1985; Flett et al. 1980). Denitrification in the lake averaged 1.4% of added nitrogen per year, too low to be significant in mass-balance calculations (Chan and Campbell 1980). In 1975, the return of phosphorus from sediments was found to be negligible in comparison to external sources. Nitrogen return from sediments was higher (Schindler et al. 1977). However, from the mid 1970's onward, concentrations of both phosphorus and nitrogen in the water column increased (Fig. 1 and 2), prompting us to investigate the possibility that a change in the sediment – water equilibrium was caused by long-term fertilization. This paper reports the results of that investigation.

Description of the Lake

Lake 227 has a surface area of 5.00 ha, a mean depth of 4.4 m, and a maximum depth of 10.0 m (Brunskill and Schindler 1971). Its terrestrial drainage is 29.4 ha[1], and contains bedrock ridges and thin tills (usually less than 0.5 m thick). The basin is covered by mature forests of *Pinus banksiana* and *Picea mariana*. There are no inflowing lakes or streams. The outlet is a discrete channel at the northwestern corner of the lake, draining over a v-notch weir. The lake stratifies strongly in summer, with a thermocline at 2–4 m. Since fertilization began, the depth of the thermocline has decreased by 1–2 m, and the duration of anoxia has increased. Spring turnover is usually incomplete below 7 m, and fall turnover is seldom complete below 9 m. Anoxic conditions prevail in the hypolimnion for much of the year (Schindler 1971 and unpublished). The average water renewal time (τ_w) for 1970–74 was 2.3 years. For the much drier period 1975–82, it averaged 4.1 years (K. Beaty, Freshwater Institute, Winnipeg, MB., 1981 and pers. comm.). Upper sediment layers have 95% water content and contain $>$50% organic matter. They are dark in colour and highly flocculent. Visible laminar structure appears in sediments deposited since 1974 (Schindler and Davidson, unpublished data).

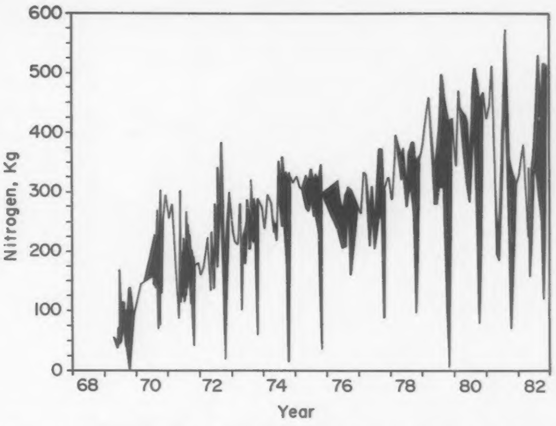

FIG. 2. The mass (kg) of nitrogen in Lake 227, 1968–82. The upper line is the whole-lake mass, the shaded area the mass in the epilimnion.

Methods

Beginning in June, 1969, the lake was fertilized with NaNO$_3$ and either Na$_2$HPO$_4$ (1969) or H$_3$PO$_4$ (1970 and thereafter) (Schindler et al. 1971, 1973). Fertilizer was added in 20 or 21 weekly increments, from mid-May until early October from 1970 onward (Schindler et al. 1971, 1973; Cruikshank 1984). The mass ratio of N:P was 14:1 in 1969–74, and 5:1 from 1975 onward (Schindler 1977).

Samples were taken from 4 to 11 depths between 1 and 10 m, at intervals of 1–4 wk during the ice-free season and 4–6 wk during the ice-covered season. Samples were filtered through Whatman GF/C filters and analyzed the same day for total dissolved phosphorus (TDP), NO$_3^-$, NH$_4^+$, and total dissolved nitrogen (TDN). The filters were frozen for later analysis of suspended phosphorus and nitrogen (SP and SN) at the Freshwater Institute in Winnipeg. An aliquot of unfiltered sample was acidified and analyzed for sodium at the Freshwater Institute by atomic absorption analyses. The methods of Stainton et al. (1977) were used for all analyses. Total phosphorus (TP) was calculated as the sum of TDP and SP; total nitrogen (TN) as TDN + SN.

Water samples for algal identifications and counts were taken at 1–2-wk intervals. At each depth, a 100 mL aliquot of lake water was preserved in acid Lugol's solution, and counted on an inverted microscope. A more detailed description is given by Schindler et al. (1971, 1973) and Findlay and Saesura (1980).

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[1] Based on recent detailed reconnaissance. Earlier papers used a basin area of 26.8 ha.

Annual inputs of all elements to the lake (I) were calculated as the sum of runoff, precipitation, and fertilizer additions. Runoff values were extrapolated from the NW subbasin of Lake 239, which was quite similar in topography and vegetation prior to a 1980 forest fire in the Lake 239 watershed (Schindler et al. 1976). Recent analyses indicate that except for a slight increase in TDN, nutrient yields changed very little after the fire, and we have continued to use the basin as an index watershed (Bayley and Schindler 1987). Precipitation inputs were calculated from annual "bulk precipitation" analyses of samples from a collector near the Experimental Lakes field camp 5 km from the lake (Linsey et al. 1987).

Losses of elements via the outflow (O) were calculated from continuous flow records (Beaty 1981, pers. comm.) and chemical analyses of weekly epilimnion or outflow samples. Annual changes in the mass of an element in the lake (ΔM) were calculated as the difference between volume-weighted concentrations on January 1 of successive years. The volume-weighted concentration on January 1 was calculated as the time-weighted average of the final sampling in a calendar year (usually early to mid-December) and the first sampling of the next year (usually mid- to late January). Annual sedimentation (S) was calculated as I−O−ΔM (Schindler and Nighswander 1970).

Two possible reasons were hypothesized for the increased nitrogen and phosphorus: (1) increased return of the elements from sediments, and (2) reduced water renewal during the succession of dry years which occurred during the later 1970's and early 1980's.

Hypothesis 1 was tested directly by deploying pore water equilibrators (Hesslein 1976) in 1982, to detect changes in pore water concentrations since 1975.

Hypothesis 2 was tested with a simple one compartment mixed reactor model, which predicted the mean lake concentrations of each element by treating it as if it were a conservative substance. The model can be represented by the mass equation:

$M_t = M_{t-1} + I - O - S_d$
M_t = mass in the lake at time = t
I = input (fertilizer plus natural)
O = outflow
S_d = diffusion into (or out of) sediments

The model accuracy was tested with the long-term sodium data, then used to investigate the less than conservative behavior of nitrogen and phosphorus.

Because hydrologic data (integrated outflow) and natural input estimates were available on a calendar year basis, the model was started on 1 January 1969 and calculations were done with a yearly time step. Values of M_t for 1 January 1969 were estimated from pre-fertilization data which had been quite constant. However, because fertilization in each year did not begin until May 1, we believed that the year-to-year behavior of Na, N, and P could be better estimated by using a weighted average of 1/3 of the starting January 1 and 2/3 of the following January 1 in each year in the modelling procedure. Much of the outflow in each year also occurred during snowmelt, prior to the major fertilizer additions.

Sediment diffusion losses or gains were estimated by a simple planar diffusion model, forced by a step-function approximation of the concentration in the lake. Concentrations in the sediment pore waters were estimated using the complementary error function (Carslaw and Jaeger 1967, p. 63). Because most of the change in lake concentration occurred during the first few years, the sediment term is most important during that period. Although in general the sediment pore waters of ELA lakes may have higher Na than in the water column at ELA (Lerman and Brunskill 1971), this results from diagenetic sediment production of Na, which we believed would be unaffected by the experiment, so that despite increasing concentrations of Na in the lake water, once a new steady-state was reached the gradient of Na from water to sediments would remain unchanged.

TABLE 1. Sources and sinks for sodium in Lake 227, January 1970 until December 1982. Precipitation data are from the main ELA collection site on Lake 239, and runoff data are calculated by assuming a yield ha^{-1} similar to the Northwest Subbasin of Lake 239. Sediment values are for pore water only. Concentrations were uniform for the entire half meter of sediment depth, but increased between 1975 and 1982. No deeper profiles were taken. It was therefore assumed that pore water concentrations were uniform to 0.75 m. Actual amounts of sodium in sediments are probably larger, but are unlikely to affect the overall budget by more than 2%. Data are in kg.

Fertilizer	4048
Precipitation	85
Runoff	918
Total input	5051
Outflow	4340
Δmass in lake	447
Sediments, top 0.75 m	327
Total "sinks"	5114
$\frac{\text{Sinks}}{\text{Input}}$, %	101

After the model was verified for sodium, results were generated for nitrogen and phosphorus to test the conservativeness of their behavior. In the case of sodium, outflows were calculated as the product of the measured concentrations in the outflow and the weekly outflow volumes. The fraction of nitrogen and phosphorus in the water mass of the lake lost via the outflow was assumed to be the same as that for sodium, because fertilization distributed all three elements identically in time and space.

Sediment diffusion was handled as for sodium. Given the history of TDN and TDP, we calculate that the sediment pore waters in the 0−75 cm layer would increase in average concentration by only:

$$\text{TDN } 0.6 \text{ mg}\cdot\text{L}^{-1}$$
$$\text{TDP } 10\,\mu\text{g}\cdot\text{L}^{-1};$$

in the top 20 cm of sediment it would be 50% higher or:

$$\text{TDN } 1.2 \text{ mg}\cdot\text{L}^{-1}$$
$$\text{TDP } 20\,\mu\text{g}\cdot\text{L}^{-1}.$$

These amounts are insignificant when compared to seasonal variance in concentration in the lake or core-to-core variances; their effect on model output is insignificant.

The assumption that sodium was conservative was verified independently of the model in three ways. Firstly, there has been no tendency for the concentration of sodium in the hypolimnion to increase during summer stratification, which would be expected if it were removed biologically from the epilimnion and released when falling particles decomposed in the

TABLE 2. The annual phosphorus budget for Lake 227 since fertilization began. ΔM is the change of mass of phosphorus in the water column from January 1 until December 31. Values marked "est" prior to fertilization are estimates derived from long-term averages for unfertilized lakes of the area. The horizontal line between 1974 and 1975 indicates when the N:P ratio in fertilizer was decreased. All values are in kg, except where indicated.

Year	Input (I)	Outflow (O)	$\frac{O}{I}$, %	Retention (R)	$\frac{R}{I}$, %	ΔM	Average mass in lake (M)	Sedimentation (S)	$\frac{S}{I}$, %
Pre-fert	1.0(est.)	0.2(est.)	—	0.8(est.)	—	0.0	3.0(est.)	0.8(est.)	80
1969	22.0	—	—	—	—	1.3	4.2	—	—
1970	31.3	3.0	10	28.3	90	2.4	6.0	25.9	83
1971	32.6	3.4	10	29.2	90	−3.1	8.3	32.4	99
1972	29.9	3.3	11	26.6	89	2.8	7.8	23.8	80
1973	30.4	3.8	13	26.6	88	−0.6	9.0	27.1	89
1974	30.5	1.9	6	28.6	94	0.3	8.4	28.3	93
$\bar{X}$ and SD									
70–74	30.9±1.1	3.1±0.7		27.9±1.2		0.4±2.4	7.9±1.1	27.5±3.2	89±7
$\bar{X}$, % of input		10		90		1	43	89	
1975	28.4	1.7	6	26.7	94	−1.5	6.4	28.2	99
1976	27.9	0.8	3	27.1	97	5.5	7.0	21.6	77
1977	28.5	2.0	7	26.5	93	2.3	10.6	24.2	85
1978	28.7	3.0	10	25.7	90	0.4	13.9	25.3	88
1979	28.8	2.2	8	26.6	92	−4.0	14.6	30.5	106
1980	28.0	0.8	3	27.2	97	8.5	17.0	18.7	67
1981	28.2	2.8	10	25.4	90	−8.2	15.3	33.6	119
1982	28.6	3.0	10	25.6	90	2.5	12.0	23.1	81
$\bar{X}$ and SD									
75–82	28.4±0.3	2.0±0.9		26.4±0.7		0.7±5.3	12.1±3.9	25.6±4.9	90±17
$\bar{X}$, % of input		7		93		2	26	91	

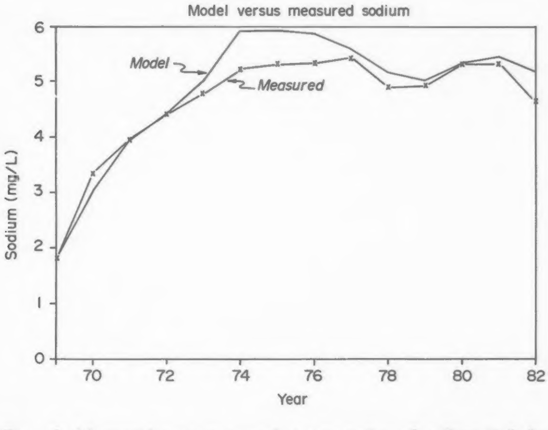


FIG. 3. Measured average annual concentration of sodium in Lake 227, compared to theoretical values calculated from the model. The theoretical values are calculated using annual water renewal values. Sodium inputs in 1975–82 were much lower due to decreased input of sodium nitrate fertilizer, as explained in the text.

hypolimnion. Secondly, summing sediment diffusion, measured outflows and increases in the concentration of sodium in the lake since fertilization began accounts for 101% of the added fertilizer (Table 1). Thirdly, average annual sodium concentrations in the lake have closely followed the concentrations predicted from annual mean water renewal rates (Fig. 3), suggesting that not only is sodium conservative, but that the strong thermal stratification of the lake is relatively unimportant in affecting its mean concentration. If fertilizer were the dominant source of all three elements throughout the study period, the long-term ratios of phosphorus and nitrogen to sodium should remain constant over time. Increases in the ratio with time would suggest increases either in internal cycling or unmeasured external sources of nutrients with time.

Results

Elemental Mass Balances

Annual budgets revealed high net fluxes of phosphorus into sediments throughout the study period, despite the hypolimnetic anoxia (Table 2). The average sedimentation rate was 89% of phosphorus input in 1970–74, and 91% in 1975–82. Total retention by the lake was only slightly higher, 90 and 93% of input. The average annual increase in phosphorus in the water column, ΔM, was only 1–2% of input.

Sedimentation of nitrogen was a lower proportion of input, and was somewhat more erratic. In one year (1982), the apparent net flux was actually from sediments to water. While the efficiency of sedimentation in 1975–82 (55%) was lower than 1970–74 (66%), these values were not significantly different, due to year-to-year variation. Retention of nitrogen averaged 79 and 62% of I for the two periods and ΔM averaged 12 and 8% of input (Table 3).

In contrast, sedimentation of sodium was very low. The positive figure (13%) from 1970 to 1974 may represent diffusion into sediment, while the negative value for 1975–82 (−2%) is of no consequence. ΔM increased during the earlier period (21%), but actually decreased in 1975–82 (−13%), due to the reduced additions of fertilizer (Table 4).

TABLE 3. The annual nitrogen budget for Lake 227 since fertilization began. ΔM is the change of mass of nitrogen in the water column. Values marked "est" are estimates derived from long-term averages for unfertilized lakes of the area. The horizontal line between 1974 and 1975 indicates when fertilization with nitrogen was decreased from an N:P by weight of 14 to an N:P of 5. All values are in kg, except where indicated.

Year	Input	Outflow	Retention	ΔM	Average mass in lake (M)	Sedimentation (S)	$\frac{S}{I}$, %
Pre-fert	56(est.)	—	—	—	—	—	—
1969	310	—	—	—	96	—	
1970	392	123	269	170	184	99	25
1971	383	96	287	−102	194	389	102
1972	369	76	292	98	227	195	53
1973	362	64	298	−11	241	309	85
1974	362	72	290	58	285	233	64
$\bar{X}$ and SD							
70–74	363 ± 29	86 ± 24	287 ± 11	43 ± 104	226 ± 40	245 ± 110	66 ± 30
$\bar{X}$, % of input		24	79	12	205	67	
1975	163	70	94	−13	258	107	66
1976	149	39	110	−31	256	141	95
1977	166	51	116	26	294	89	54
1978	161	83	79	75	342	4	2
1979	176	73	103	18	399	86	49
1980	160	37	124	46	418	77	48
1981	173	61	112	−117	380	229	132
1982	187	96	91	104	397	−13	−7
$\bar{X}$ and SD							
75–82	167 ± 12	64 ± 20.8	104 ± 15	14 ± 69	343 ± 66	90 ± 76	55 ± 45
$\bar{X}$, % of input		38	62	8	62	54	

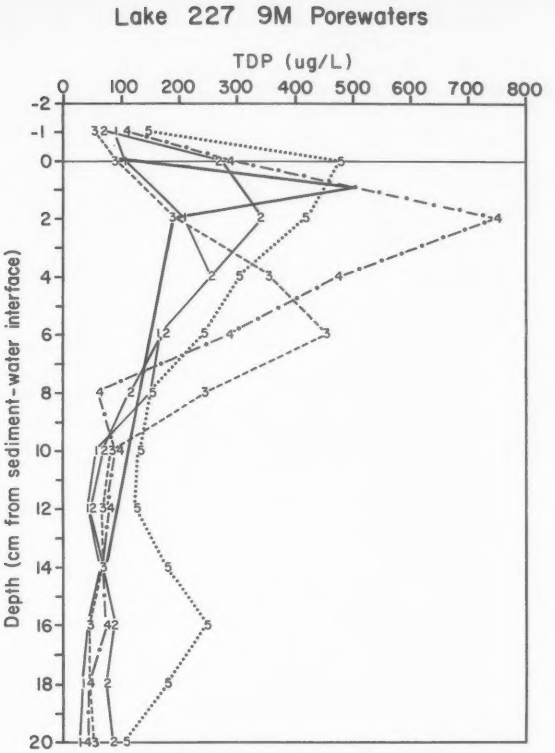
TABLE 4. The annual sodium budget for Lake 227 since fertilization began. ΔM is the change of mass of sodium in the water column from January 1 until December 31. The horizontal line between 1974 and 1975 indicates when the N:P ratio in the fertilizer was decreased. All values are in kg, except where indicated.

Year	Input (I)	Outflow	Retention	ΔM	Average mass in lake (M)	Sedimentation (S)	$\frac{S}{I}$, %	Water renewal (yr)
1969	505	—	—	—	393	—		—
1970	626	428	198	233	740	−35	−6	1.42
1971	658	469	189	75	877	114	17	1.91
1972	576	361	215	237	975	68	12	2.64
1973	581	390	191	−7	1059	198	34	2.63
1974	586	364	222	183	1154	39	7	2.91
$\bar{X}$ and SD	605	402	203	126	866	77	13	2.30
70–74	35	46	15	94	273	87	15	0.62
$\bar{X}$, % of input		66	34	21	143	13		
1975	247	330	−83	−177	1174	94	38	2.87
1976	226	179	47	203	1178	−156	−69	5.56
1977	236	333	−97	−139	1200	42	18	3.19
1978	245	430	−185	−97	1083	−88	−36	2.24
1979	240	264	−24	94	1090	−118	−49	4.00
1980	263	140	123	55	1176	68	26	8.53
1981	284	282	2	−104	1180	102	36	4.03
1982	284	370	−86	−108	1021	22	8	2.55
$\bar{X}$ and SD	253	291	−38	−34	1138	−4	6	4.12
75–82	22	96	96	134	64	101	41	2.07
$\bar{X}$, % of input		115		−13	450	−2		

Pore Water Profiles

Concentrations of phosphorus in pore waters did not increase between 1975 and 1982 (Fig. 4). There is, therefore, no reason to modify the conclusions of Schindler et al. (1977), that return of phosphorus from sediments is of minor consequence in the lake.

In contrast, the concentrations of ammonium in pore waters increased by 2 $mg \cdot L^{-1}$ between 1975 and 1982 (Fig. 5). However, concentrations in overlying water increased by a



Values above sediment-water interface represent means of values observed in overlying water

FIG. 4. Profiles of total dissolved phosphorus in sediment pore waters at a depth of 9 m in Lake 227. Concentrations were determined using the close interval pore water sampler of Hesslein (1976). The solid line is 30 May 1975. 1 and 2 are separate profiles on 8 June 1982; 3 --- is 21 July 1982; 4 — • — 19 Aug. 1982; 5 . . . . is 15 Sept. 1982.

similar amount, so that the diffusive return of nitrogen calculated by Fick's law was similar to that obtained in 1975 (Schindler et al. 1977).

Modelling

The model slightly underestimated the conservativeness of sodium in the lake water in the first few years, and was in near perfect agreement subsequently (Fig. 6). This may be due to imperfect sediment modelling, which was more important in the early years when there was the greatest rate of change in concentration in the lake. The results for nitrogen and phosphorus showed a slight decline in apparent conservativeness from 1969 to 1975−76, of similar magnitude to that for sodium. After 1976, values for phosphorus continued to parallel those for sodium, indicating little or no change in sediment − water exchange.

In contrast, the increase in conservativeness of nitrogen increased with time after 1976. This indicates a significant increase in some source of nitrogen, which will be discussed below.

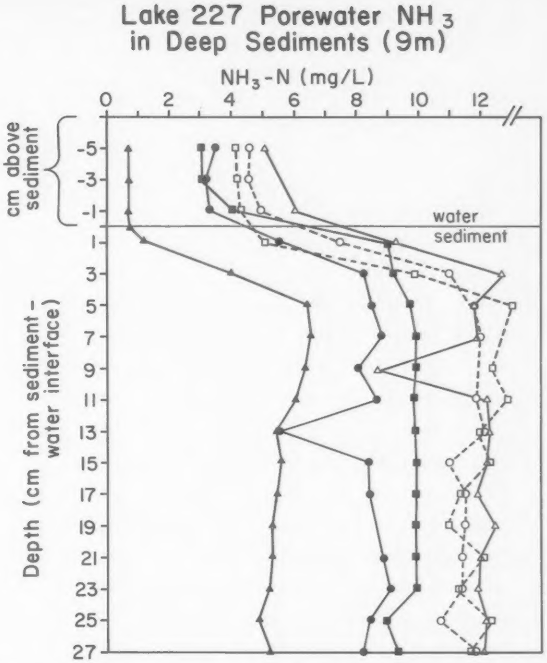


FIG. 5. Concentrations of ammonium nitrogen in sediment pore waters at a depth of 9 m in Lake 227. Samples were taken using the close interval pore water sampler of Hesslein (1976) and analyzed according to Stainton et al. (1977). In 1975, total dissolved nitrogen concentrations were only slightly higher than NH_4-N, indicating that the latter is the dominant form of nitrogen in pore water. — ▲ = June 1975, — ● = 8 June 1982; -- ■ = 21 July 1982, -- ○ = 19 Aug. 1982; -- Δ = Sept. 1982.

Discussion

The mass-balance model indicates that increased concentrations of phosphorus observed in the lake are caused by slower water renewal rates, and not by return from sediments. This is confirmed by pore water results. Similar conclusions were reached by Levine et al. (1986) from additions of $^{32}PO_4$ to the whole lake. They found that the maximum rate of phosphorus return from sediments was less than 4% of input. These results confirm that there has been no loss in efficiency of phosphorus sedimentation over 15 yr of eutrophication, even though the lake has been anoxic below 7 m for 10−12 months of the year.

Schindler et al. (1977) hypothesized that dilution of phosphorus-rich sedimenting material by mixing of near-surface sediments was responsible for the lack of significant phosphorus return. A change in sediment characteristics since 1975 now makes this explanation seem improbable. A freeze-core taken in 1982 clearly shows the mixed character of deep sediments prior to 1974, but distinct annual laminae since that time can be confirmed by the sequence of pollen and diatom remains in sediments (Schindler and Davidson, unpublished data). We do not know the reason why sediment mixing ceased at precisely the time when nutrient ratios were changed, but suspect that it is due to the stabilizing effect of sedimented

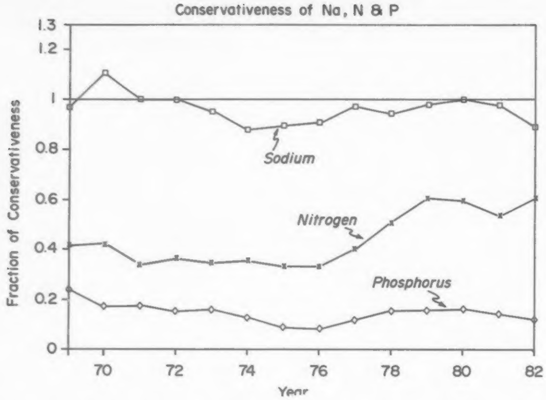


FIG. 6. Results of the simple model of "conservativeness" for sodium, nitrogen and phosphorus as calculated from equation 1. A value of 1.0 would be perfectly conservative.

layers of blue-green algal filaments, which now occur annually. As the result of the cessation of sediment mixing, surface sediments now have phosphorus concentrations within 20% of those expected from known rates of phosphorus sedimentation and sediment accumulation. It thus seems unlikely that sediments will ever release significant amounts of phosphorus at the present rate of phosphorus input.

The model indicates that increased concentrations of nitrogen are not simply due to decreased water renewal. However, calculated fluxes from sediment pore water profiles indicate that these have not changed in recent years, because increases in pore water concentrations have been matched by increases in the concentration in overlying water. The most likely source of the nitrogen therefore seems to be increased nitrogen fixation, which was not included in inputs in Table 3, as mentioned earlier.

In 1975, nitrogen-fixation accounted for 14% of the total nitrogen input, based on conversion of acetylene reduction results to nitrogen using a 3 : 1 ratio (Flett et al. 1980). While N_2 fixation was not measured in later years and blooms of nitrogen-fixing blue-green algae have been sporadic, there has been a general tendency for blooms to become much larger suggesting that the importance of the atmosphere as a source of nitrogen to the lake has increased. The apparent net backflux of nitrogen from sediments in 1982 may therefore be an indication of vastly underestimated nitrogen inputs, because the largest bloom of nitrogen-fixing blue-green algae recorded in the lake occurred that year (Schindler 1985).

One feature of the nutrient budgets merits additional mention. Sedimentation (S) of nutrients is usually assumed to be a function of mass of the nutrient in a lake ($\overline{M}$) (Vollenweider 1976). However, annual sedimentation of both phosphorus and nitrogen in Lake 227 were more closely correlated with input than with mean mass (Tables 2 and 3). Correlation coefficients between I and S were 0.41 and 0.65 for P and N, respectively. Both values are highly significant. Corresponding correlations between $\overline{M}$ and $\bar{S}$ were −0.04 and −0.54. The *r* value for phosphorus is not significant.

In summary, after 15 years of fertilization, both sodium and phosphorus concentrations appear to be in equilibrium with inputs of these elements, and there have been no major changes in the cycle of either element. Due to the lack of mixing after 1974, surface sediments now appear to be rapidly approaching equilibrium with phosphorus inputs. Nevertheless, concentrations of phosphorus in the water are still governed by input, water renewal, and sedimentation of plankton. In contrast, even after such a long period of stable inputs, neither nitrogen fixation nor nitrogen concentrations in pore waters have stabilized. Concentrations of nitrogen in the lake continue to increase, apparently due to increased nitrogen fixation. This work was supported by the Canadian Department of Fisheries and Oceans.

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Phytoplankton Community Responses to Nutrient Addition in Lake 226, Experimental Lakes Area, Northwestern Ontario

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Inclusion of phosphorus in fertilizer added to one of two basins of a small lake, between which water exchange was greatly reduced by a vinyl sea-curtain, significantly increased epilimnetic phytoplankton biomass, and altered species composition. Over an 8-yr period, the average biomass of phytoplankton in the basin receiving carbon, nitrogen, and phosphorus (in ratios of 10:5:1) increased 4 to 8 times and the biomass in the basin receiving only carbon and nitrogen (in ratios of 10:5) increased 2 to 4 times over non-fertilized years. The basin receiving all three nutrients consistently had blooms in late summer which were dominated by nitrogen fixing species of cyanophytes. In comparison with unfertilized reference lakes, the proportion of phytoplankton in fertilized basins which was available to zooplankton as food was 10 times greater in the basin receiving all three nutrients, and two times greater in the basin receiving only nitrogen and carbon. When fertilization of both basins was stopped, species composition and levels of biomass reverted within a year to the composition and biomass levels of phytoplankton observed in the reference lakes and remained at those levels for 2 further years of study.

L'addition de phosphore au fertilisant ajouté dans l'un des deux bassin d'un petit lac où les échanges d'eau étaient fortement limités par un rideau de vinyle s'est traduite par une augmentation significative de la biomasse du phytoplancton épilimnétique et par une modification de la composition des espèces. Au cours d'une période de 8 années, la biomasse moyenne du phytoplancton dans le bassin fertilisé par du carbone, de l'azote et du phosphore (rapport de 10:5:1)s'est accrue de 4 à 8 fois tandis que celle du bassin fertilisé seulement par du carbone et de l'azote (rapport 10:5) ne s'est accrue que de 2 à 4 fois par rapport aux années où il n'y a pas eu fertilisation. D'importantes croissances d'algues, dominées par des espèces de cyanophytes fixant l'azote, apparaissaient à fin de chaque été dans le bassin fertilisé par les trois matières nutritives. Comparativement aux lacs témoins non fertilisé, la proportion du phytoplancton des bassins fertilisés pouvant servir de nourriture au zooplancton était 10 fois supérieure dans le bassin fertilisé par les trois matiéres nutritives et deux fois supérieure dans le bassin fertilisé que par de l'azote et du carbone. Après la fin de la fertilisation, la composition en espèces et les volumes de biomasse des deux bassins sont revenus, en moins d'un an, aux valeurs des lacs témoins et sont demeurés à ces niveaux pendant les 2 autres années de l'étude.

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During the late 1960's and early 1970's eutrophication was recognized as a major environmental problem. Research focussed on identifying which of the primary nutrients — carbon, nitrogen, or phosphorus — was limiting and then to determining a means of controlling the limiting nutrient. Based on laboratory culture work, several investigators (Lange 1967, 1970, 1971; Kuentzel 1969; Kerr et al. 1970) concluded that carbon was the limiting nutrient. On the basis of a whole-lake experiment, Schindler et al. (1973) showed that even at low levels of dissolved carbon, algal blooms could be stimulated by additions of phosphorus and nitrogen. In a whole-lake experiment on Lake 226 (Schindler 1974), the role of phosphorus in stimulating algal blooms was explored. A small double-basin lake, in the Experimental Lakes Area (E.L.A.) of northwestern Ontario, was divided in half with a vinyl sea-curtain. Carbon, nitrogen, and phosphorus were added to the northeast basin and only carbon and nitrogen to the southwest basin. Weekly additions were made during the open water season from 1973 to 1980. Early results of the experiment were described by Schindler (1974).

The pupose of this paper is to present the responses of the phytoplankton community to the continued fertilization in each of the Lake 226 basins. Biomass, species composition, changes in the percentage of edible food, and response of cyanophytes to N:P ratios, will be discussed for 8 consecutive years of fertilization and for 3 yr of recovery. In addition to the experimental system Lakes 224 and 239 have been chosen to serve as reference basins. These lakes have not been experimentally manipulated and have been monitored for the same period.

Methods

Study Lakes

The Experimental Lakes Area is located in northwestern Ontario on the Canadian Shield (Brunskill and Schindler 1971; Cleugh and Hauser 1971). The lakes are situated on Precambrian granite and the surrounding watersheds are typical boreal forest of pine and spruce.

TABLE 1. Morphometric and fertilizer data for study lakes.

Lake	Surface area (ha)	Maximum depth (m)	Mean depth (m)	Volume ($10^5 m^3$)	Mean water residence time[a] (1973–83) (yr)	Annual fertilizer inputs			
						Sucrose (g C·m^{-2})	Sodium nitrate $NaNO_3$ (g N·m^{-2})	Phosphoric acid H_3PO_4 (g P·m^{-2})	Ratio C:N:P
226SW	7.8	10.0	6.3	4.9	6.9	3.67	1.92	none	10:5:0
226NE	8.3	14.0	5.7	4.7	3.3[b]	3.45	1.81	0.34	10:5:1
224	25.9	27.4	11.6	30.1	62.8[c]	none	none	none	
239	59.1	30.0	10.5	56.1	8.0	none	none	none	

[a] Computed from data in Beaty 1981, 1984.

[b] Lake 226 mean water residence time is 6.5 yr.

[c] Mean for 1976–80, 1982–83. Groundwater loss is not measured and therefore the value is an upper limit.

Lake 226 is a small dimictic double-basin lake which was classified as oligotrophic before fertilization began. The two basins (northeast (NE) and southwest (SW)), are connected by a narrow channel 50 m wide and 3 m deep. Natural flow is from south to north with a single outflow located at the north end of the northeast basin. There are no stream inflows into either basin. The basins are similar in morphometry (Table 1). In 1973 the basins were separated by installing a vinyl sea-curtain in the connecting channel and nutrient additions to both basins began (Table 1). During the summer of 1979 a forest fire burnt the north side of the lake along the full length of both basins, destroying 34% of the forest cover (Schindler et al. 1980; Beaty 1984).

Although the reference Lakes 239 and 224 are morphometrically different from Lake 226 (Table 1), their chemistry is representative of unperturbed lakes of this area (Prokopowich 1979) and their phytoplankton assemblages were similar to those of Lake 226 before fertilization. The watershed surrounding Lake 239 has been subjected to several natural disasters. In 1973 a major windstorm caused extensive blowdown and in 1974 and 1980 forest fires burnt 70.8 and 100% of the watershed, respectively (Schindler et al. 1980; Beaty 1984).

Fertilization

Fertilization of Lake 226 was carried out in equal weekly increments during the ice-free seasons from 1973 to 1980. From 1973 to 1974 the fertilizer was mixed into the epilimnion by the prop wash of a boat. In 1975 the method of addition was changed to a semi-continuous trickle feed from a barrel to simulate a point source addition. Sucrose ($C_{12}H_{22}O_{11}$), sodium nitrate ($NaNO_3$) and phosphoric acid (H_3PO_4) were the C, N, and P sources, respectively. Lake 226 southwest received annual additions of carbon (3.67 g·m^{-2}) and nitrogen (1.92 g·m^{-2}) while the northeast basin received similar additions of carbon and nitrogen but also phosphorus (0.34 g·m^{-2}) (Table 1). The fertilizer was added to the epilimnion at a N:P ratio of 5:1, by weight. For a complete description of the method of fertilizing and the types of chemicals used see Cruikshank (1984). The study continued for 3 further years (1981–83) during which no nutrients were added.

Phytoplankton Sampling and Enumeration

During the course of the experiment two sampling techniques for phytoplankton were used. From 1972 to 1975, discrete samples were taken with a 2-L Vandorn bottle, from odd meter depths to a maximum of twice the secchi disk depth. From 1975 to 1983, samples were taken in conjunction with the primary productivity program. These samples were obtained with an integrating sampler (Shearer 1978). A 1.5-L opaque bottle with a stopper and trip mechanism was used to obtain an integrated sample from the desired stratum (epilimnion, metalimnion, or hypolimnion). After lowering the sampler, it was opened by means of a messenger, and as the bottle filled, it was lowered and raised through the desired stratum. The upper and lower boundaries of the strata were predetermined from light, temperature and chlorophyll profiles to a maximum depth at 0.5% of surface irradiance.

Samples of phytoplankton were taken approximately once every 2 wk during the ice-free season. Lake 226 was sampled in 1971 and 1973 to 1983. Lake 239 was sampled from 1971 to 1983 and Lake 224 was sampled from 1974 to 1977 and 1980 to 1983. In the field lab a 125 mL aliquot was extracted and preserved in Lugol's solution for identification and enumeration of phytoplankton. To aid in identification, live net samples were examined occasionally.

For counting and identification, 10–20 mL aliquots were allowed to settle from 12 to 24 h, depending on the phytoplankton density. The samples were then examined through a Zeiss inverted microscope. Counts were performed at magnifications of 125×, 400×, and 1 000× with phase contrast illumination. All counts were done by the Utermohl technique as modified by Nauwerck (1963).

For the purpose of estimating phytoplankton wet weight biomass it was necessary to approximate the cell volume of each species. Estimates of volume for individual cells were obtained by applying the geometric formula best fitted to the shape of the cell (Vollenweider 1968). A density of 1.0 was assumed in calculating cell wet weight.

Duplicates for each sample were counted from 1975 to 1983 and the data were then averaged. To compare duplicate counts we used a similarity index:

$$\% \text{ similarity } = \sum_{i=1}^{s} \min\,(P_{1i},\ P_{2i})$$

where s = total number of species in both samples

P_{1i} = proportion of the total biomass, of species i in sample 1.

P_{2i} = proportion of the total biomass, of species i in sample 2.

The computation favors the dominant species so that changes in less abundant groups are not obvious. Over 9 yr the sim-

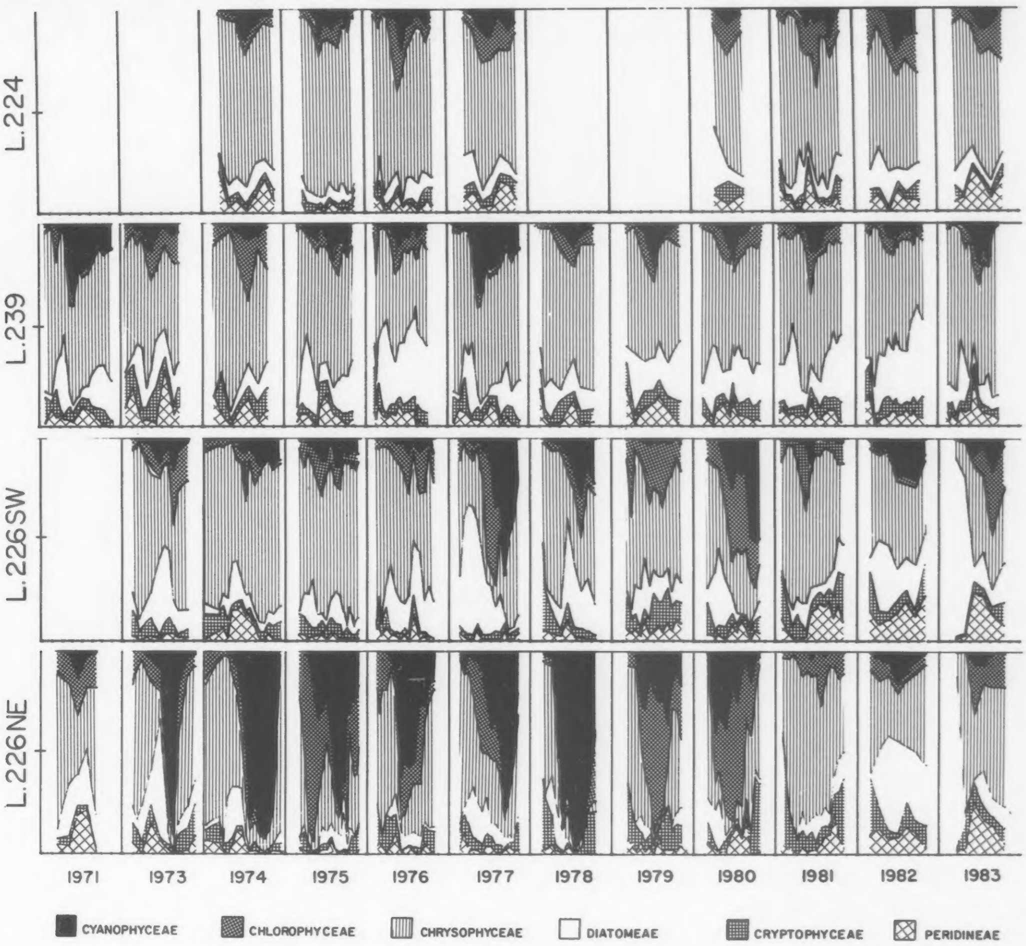

FIG. 1. Accumulative percent phytoplankton composition (computed from biomass (mg·m^{-3})) for ice-free seasons 1971–83 for reference Lakes 224 and 239 and for Lake 226 northeast (C, N, and P additions) and Lake 226 southwest (C and N additions).

ilarity between duplicate counts ranged from 85 to 93% in all lakes.

Edible Portion of Biomass

Since phytoplankton play a major role in the food web of lakes we attempted to assess the abundance of food available to higher trophic levels, such as zooplankton and benthos.

Our criteria for edibility by zooplankton was any phytoplankton cell with a maximum dimension of less than 20 μm. This is a conservative criterion compared to that in the literature, and therefore the estimates of edible biomass (mg·m^{-3}) are, if anything, underestimates. However, increasing the cutoff point to 35 μm changed the computed annual edible biomass by less than 10%. We also excluded from the edible fraction, pennate diatoms and filamentous blue greens not considered to be edible by zooplankton (Burns 1968; Porter 1973, 1977).

The diverse benthic communties in E.L.A. lakes are dominated by dipterans, including both filter feeding and raptorial feeding species (Ian Davies, Freshwater Institute, pers. comm.). All sizes of algal particles will presumably be consumed by such communities since cells that are too large to be processed by grazers will be consumed by filter feeders as a biproduct of bacterial degradation. Armitage (1968) and Moore (1979) showed that Diptera are impartial to the type of food source.

Principal Component Analysis

Changes in the composition of phytoplankton communities were assessed by principal component analysis (SAS Institute Inc., 1985). The correlation matrix of percent of total biomass in the epilimnion represented by each of the seven taxonomic groups (Cyanophytes, Chlorophytes, Euglenophytes, Chrysophytes, Diatoms, Cryptophytes, and Dinoflagellates) was analyzed. Lakes 224, 239, 226 northeast, and 226 southwest were all included in the data matrix, and sampling days were restricted to the open-water season from 1971 to 1983.

TABLE 2. Major species found in each year for all four lakes in order of abund
5 — Diatoms, 6 — Cryptophytes, 7 — Peridineae.

	Reference Lakes		
	239	224	SW ba
1971	4 large Chrysophyceae[a] 4 *Dinobryon bavaricum* var. *Vanhoeffenii* (Bach.) Kreiger 5 *Rhizosolenia eriensis* H. L. Smith 4 *Dinobryon bavaricum* Imhof 1 *Merismopedia glauca* (Ehren.) Naeg.		
1972	4 Large Chrysophceae[a] 4 *Dinobryon sociale* Ehren. 4 *D. bavaricum* Imhof 1 *Gomphosphaeria lacustris* Chodat. 4 small Chrysophceae[a] 2 *Botryococcus Braunii* Kuetz.		
1973	4 *Dinobryon bavaricum* var. *Vanhoeffenii* (Bach) Kreiger 4 *Dinobryon sertularia* Ehren. 4 *D. bavaricum* Imhof 7 *Peridinium pusillum* (Penard) Lemm. 5 *Synedra acus* Kuetz. 5 *Cyclotella comta* (Ehren.) Kuetz. 1 *Anabaena circinalis* Raben. 4 *Stichogloea* spp. 6 *Rhodomonas minuta* Skuja		4 large 5 *Tabe* 4 *Dino* 2 *Spon*
1974	4 *Dinobryon bavaricum* Imhof 2 *Botryococcus Braunii* Kuetz. 4 *Chrysochromulina* spp.	2 *Botryococcus Braunii* Kuetz. 7 *Gymnodinium* sp. 4 *Dinobryon bavaricum* Imhof	7 *Peria* 4 *Dino* 4 *D. c* 4 *Chry* 4 *Dino* 4 *Chry*
1975	4 large chrysophyceae[a] 5 *Rhizosolenia eriensis* H. L. Smith 4 *Dinobryon bavaricum* Imhof	4 *Dinobryon cylindricum* Imhof 4 *D. bavaricum* var. *Vanhoeffenii* (Bach) Kreiger 4 large Chrysophyceae[a] 2 *Botryococcus Braunii* Kuetz.	
1976	5 *Rhizosolenia eriensis* H.L. Smith 4 *Uroglena americana* Calkins 5 *Tabellaria fenestrata* (Lyngbya) Kuetz.	2 *Botryococcus Braunii* Kuetz. 4 *Dinobryon bavaricum* var. *Vanhoeffeni* (Bach) Kreiger 4 *Uroglena americana* Calkins 4 *Chrysochromulina* spp.	4 *Synu* 4 large 4 *Dino* 5 *Cycl*
1977	4 *Uroglena americana* Calkins 5 *Tabellaria fenestrata* (Lyngbya) Kuetz. 4 large Chrysophyceae[a] 5 *Dinobryon bavaricum* Imhof	4 *Chrysochromulina parva* Lackey 4 *Dinobryon bavaricum* Imhof 1 *Chroococcus limneticus* Lemm. 4 large *Chrysophyceae*[a] 4 *Chrysochromulina* spp.	2 *Spon* 4 *Dino* 1 *Oscil* 5 *Rhiz* 1 *Anab* 2 *Teili*

abundance based on biomass ($mg \cdot m^{-3}$). 1 — Cyanophytes, 2 — Chlorophytes, 4 — Chrysophytes,

Treated Lake 226	
SW basin	NE basin
	4 *Dinobryon bavaricum* Imhof 2 small greens[a] 5 *Tabellaria fenestrata* (Lyngbya) Kuetz. 4 large Chrysophyceae[a] 4 small Chrysophyceae[a] 7 *Peridinium wisconsiense* Eddy 4 *Dinobryon crenulatum* W. & G.S. West
4 large chrysophyceae[a] 5 *Tabellaria fenestrata* (Lyngbya) Kuetz. 4 *Dinobryon bavaricum* Imhof 2 *Spondylosium planum* (Wolle) W. & G.S. West	1 *Anabaena spiroides* Klebs 4 large Chrysophyceae[a] 5 *Tabellaria fenestrata* (Lyngbya) Kuetz. 4 *Dinobryon sertularia* Ehren. 4 *D. bavaricum* Imhof 4 *D. divergens* Imhof
7 *Peridinium aciculiferum* Lemm. 4 *Dinobryon sertularia* Ehren. 4 *D. cylindricum* Imhof 4 *Chrysosphaerella longispina* Laut. 4 *Dinobryon bavaricum* Imhof 4 *Chrysochromulina parva* Lackey	1 *Oscillatoria Redekei* Van Goor 1 *Anabaena planctonica* Brunn. 1 *A. solitaria* f.a. *planctonica* Brunn. 1 *A. spiroides* Klebs
4 *Synura sphagnicola* Korsch. 4 large cyrysophyceae[a] 4 *Dinobryon sertularia* Ehren. 5 *Cyclotella comta* (Ehren.) Kuetz.	1 *Anabaena solitaria* f.a. *planctonica* Brunn. 6 *Cryptomonas erosa* Ehren. 4 large Chrysophyceae[a] 2 small greens[a] 4 *Synura sphagnicola* Korsch.
2 *Spondylosium planum* (Wolle) W. & G.S. West 4 *Dinobryon sertularia* Ehren. 1 *Oscillatoria limnetica* Lemm. 5 *Rhizosolenia eriensis* H.L. Simth 1 *Anabaena solitaria* f.a. *planctonica* Brunn. 2 *Teilingia granutatum* Roy et Biss.	2 *Spondylosium planum* (Wolle) W. & G.S. West 4 *Salpingoeca* sp. 4 *Dinobryon bavaricum* Imhof 4 *D. sociale* Ehren. 1 *Anabaena Levanderi* Lemm. 6 *Cryptomonas erosa* Ehren.

1978	4 *Uroglena americana* Calkins 5 *Rhizosolenia eriensis* H.L. Smith 4 *Dinobryon bavaricum* Imhof 4 *Chrysosphaerella longispina* Laut. 4 *Dinobryon sertularia* var. *protuberans* (Lemm.) Krieger		4 *Dinobryon soci* 4 large Chrysophy 5 *Cyclotella comt* 4 *Synura sphagni* 1 *Oscillatoria Re* 5 *Cyclotella glom*
1979	4 *Dinobryon bavaricum* Imhof 4 *Chrysochromulina* spp. 4 large Chrysophyceae[a] 7 *Gymnodinium* sp. 5 *Tabellaria fenestrata* (Lyngbya) Kuetz. 7 *Peridinium aciculiferum* Lemm.		2 *Chlorella* cf *mu* 2 *Spondylosium p* 2 *Dictyosphaeriu* 4 *Dinobryon bava* 4 *Chrysochromuli* 4 *Dinobryon sertu* 5 *Cyclotella glom* 4 *Salpingoeca* sp.
1980	4 *Chrysochromulina* spp. 5 *Rhizosolenia eriensis* H.L. Smith 4 *Dinobryon sertularia* (Lyngbya) Kuetz. 4 *D. sertularia* var. *protuberans* (Lemm.) Krieger 4 *Urgolena americana* Calkins	3 *Cyclotella comta* (Ehren.) Kuetz. 4 *Uroglena americana* Calkins 4 *Dinobryon bavaricum* Imhof 4 large Chrysophyceae[a]	1 *Oscillatoria Re* 4 *Dinobryon bava* 4 *Chrysochromuli* 2 *Oocystis lacustr* 4 *Synura sphagni*
1981	4 *Uroglena americana* Calkins 5 *Synedra acus* Kuetz. 4 *Dinobryon sertularia* Ehren.	4 *Uroglena americana* Calkins 4 *Dinobryon bavaricum* Imhof 2 *Gloeococcus Schroeteri* (Chodat) Lemm. 5 *Rhizosolenia eriensis* H.L. Smith 4 *Dinobryon sertularia* Ehren.	4 *Chrysochromuli* 1 *Oscillatoria Re* 7 *Peridinium acic* 5 *Rhizosolenia er* 4 *Dinobryon bava* 7 *Gymnodinium* s
1982	4 *Dinobryon sociale* Ehren. 4 *D. bavaricum* Imhof 5 *Rhizosolenia eriensis* H.L. Smith	1 *Lyngbya* sp. 4 *Dinobryon bavaricum* Imhof 2 *Botryococcus Braunii* Kuetz. 4 *Uroglena americana* Calkins 4 *Stichogloea* spp.	4 *Chrysochromuli* 1 *Oscillatoria Re* 7 *Peridinium acic* 5 *Rhizosolenia eri* 4 *Dinobryon bava* 7 *Gymnodinium* s
1983	4 *Uroglena americana* Calkins 4 *Dinobryon sertularia* Ehren. 5 *Rhizosolenia eriensis* H.L. Smith 4 *Chrysochromulina parva* Lackey	4 *Dinobryon bavaricum* Imhof 4 *Uroglena americana* Calkins 2 *Botryococcus Braunii* Kuetz. 4 *Chrysochromulina* sp. 2 *Gymnodinium* sp. 5 *Rhizosolenia eriensis* H.L. Smith 6 *Cryptomonas erosa* Ehren.	5 *Rhizosolenia lo* 2 *Teilingia granu* 1 *Lyngbya* sp. 7 *Gymnodinium* s 4 *Uroglena ameri* 4 *Chrysochromuli* 4 *Chrysochromuli*

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[a] Unable to identify past group level. Possibly more than one species making up the total.

yon sociale var. stipitatum (Stein) Lemm.	1 *Anabaena solitaria* f.a. *planctonica* Brunn.
Chrysophyceae[a]	1 *Oscillatoria limnetica* Lemm.
ella comta (Ehren.) Kuetz.	2 *Chlamydomonas* sp.
sphagnicola Korsch.	1 *Anabaena Levanderi* Lemm.
toria Redekei Van Goor	1 *Oscillatoria Redekei* Van Goor
ella glomerata Bachmann	4 *Dinobryon sertularia* Ehren
lla cf *mucosa* Kors.	1 *Oscillatoria limnetica* Lemm.
losium planum (Wolle) W. & G.S. West	1 *Anabaena solitaria* fa. *plantonica* Brunn.
sphaerium simplex Skuja	1 *Oscillatoria Redekei* Van Goor
ryon bavaricum Imhof	2 *Spondylosium planum* (Wolle) W. &. G.S. West
chromulina parva Lackey	4 small Chrysophyceae[a]
ryon sertularia Ehren.	
ella glomerata Bachmann	
goeca sp.	
toria Redekei Van Goor	2 *Oocystis lacustris* Chodat
ryon bavaricum Imhof	1 *Oscillatoria Redekei* Van Goor
chromulina parva Lackey	1 *Anabaena cylindrica* Lemm.
is lacustris Chodat	1 *Oocystis submarina* var. *lacustris* Skuja
sphagnicola Korsch.	4 *Chrysochromulina parva* Lackey
	2 *Spondylosium planum* (Wolle) W. & G.S. West
chromulina spp.	4 *Rhizosolenia eriensis* H.L. Smith
toria Redekei Van Goor	4 *Uroglena americana* Calkins
nium aciculiferum Lemm.	6 *Cryptomonas erosa* Ehren.
olenia eriensis H.L. Smith	
ryon bavaricum Imhof	
dinium sp.	
chromulina spp.	5 *Rhizosolenia eriensis* H.L. Smith
toria Redekei Van Goor	4 *Uroglena americana* Calkins
nium aciculiferum Lemm.	6 *Cryptomonas erosa* Ehren.
olenia eriensis H.L Smith	
ryon bavaricum Imhof	
dinium sp.	
olenia longiseta Zach.	5 *Rhizosolenia longiseta* Zach.
ia granutatum Roy et Biss.	4 *Synura spagnicola* Korsch.
a sp.	2 *Teilingia granutatum* Roy et Biss.
dinium sp.	7 *Gymnodinium* sp.
na americana Calkins	4 *Uroglena americana* Calkins
chromulina spp.	2 *Gloeococcus Schroeteri* (Chodat) Lemm.
chromulina parva Lackey	4 *Chrysochromulina* spp.
	4 *Chrysochromulina parva* Lackey

tal.

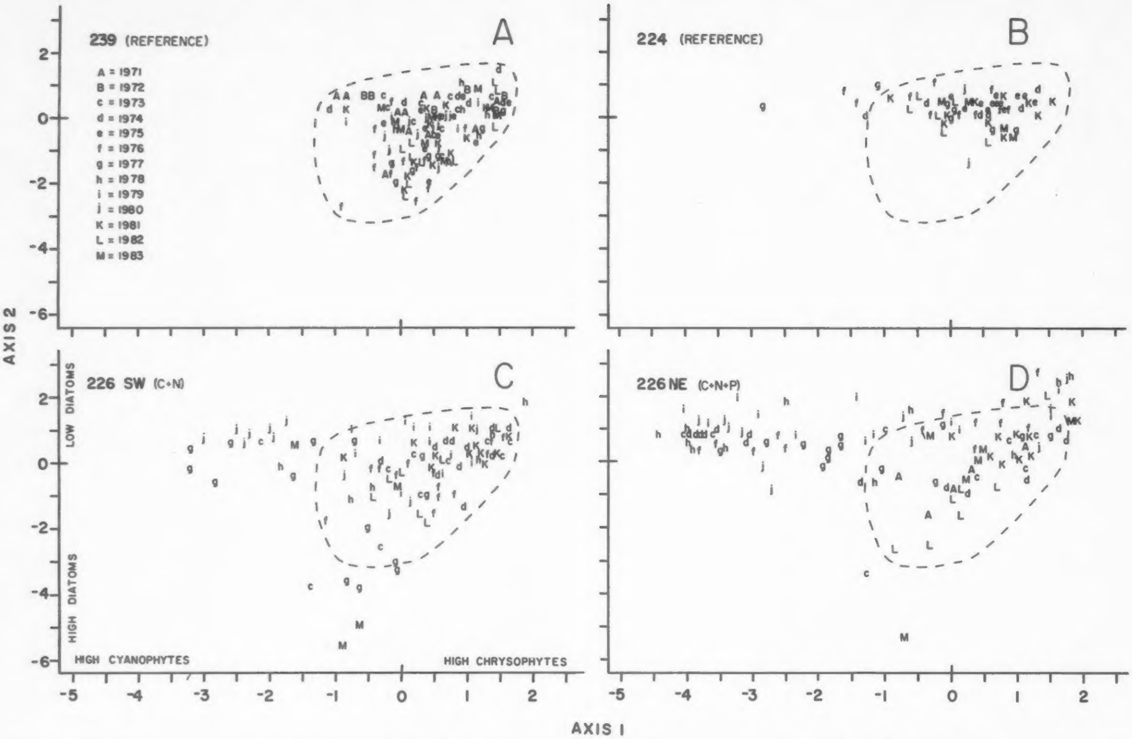

FIG. 2. The first two components from an analysis of principal components on the percentage of total phytoplankton biomass represented by each taxonomic group. Axis 1 separates samples with a high percentage of cyanophytes from those with a high percentage of chrysophytes. The second axis distinguishes samples with high percentages of diatoms from those with low percentages of diatoms. Individual sampling dates are plotted with the symbol for their respective years. Fertilization and non-fertilization years in Lake 226 are differentiated by lower and upper case letters, respectively.

Chemical Sampling and Methods

Chemical sampling for Lake 226 was done bi-weekly and monthly for the reference lakes. Samples were taken from a single station located at the maximum depth of the lake. Total phosphorus (TP) and total nitrogen (TN) were measured as described by Stainton et al. (1977). Total phosphorus and nitrogen loading rates for all lakes were computed from natural inputs (precipitation, stream inflow, and indirect runoff) and fertilizer additions as applicable.

Results

Lake 224 and 239 — Reference Lakes

The epilimnetic phytoplankton assemblages in both lakes have been dominated entirely by chrysophytes, which comprise over 50% of the total biomass throughout the period of record (Fig. 1, Table 2). The other major groups (cyanophytes, chlorophytes, diatoms, cryptophytes, and dinoflagellates) were conspicuous, but never dominant.

These observations are supported by an analysis of principal components. The major components describing phytoplankton community structure were the proportion of cyanophytes and chrysophytes (component 1 accounted for 27% of total variance) and high or low proportion of diatoms (component 2 accounted for 18% of total variance). Samples collected over 13 yr (1971–83) from both lakes 239 and 224 fall within a tight cluster in the higher chrysophyte zone with no obvious pattern through time (Fig. 2A and 2B).

Since monitoring of these lakes began there has been no detectable change in the proportion of epilimnetic biomass which is edible by zooplankton (Fig. 3). Epilimnetic phytoplankton biomass in lakes 224 and 239 has been stable throughout the period of study apart from a small increase in 1981, 1982, and 1983 (Fig. 4). Lake 224 averaged 700 ± 60 $mg \cdot m^{-3}$ ($\bar{x} \pm 1$ SD) from 1974 to 1980 and then increased 1.3× to 950 ± 30 $mg \cdot m^{-3}$ in 1981 to 1983 (Table 3). Lake 239 had a mean epilimnetic biomass of 720 ± 200 $mg \cdot m^{-3}$ from 1973 to 1980 and then increased to 1.7× to 1200 ± 40 $mg \cdot m^{-3}$ in 1981 to 1983.

The natural disasters (windstorm in 1973 and fires in 1974 and 1980) had no detectable effect on phytoplankton species composition. However, increased biomass for Lake 239 in 1981–83 could be a response to the burning of the entire watershed in 1980.

Lake 226 Southwest — Fertilized with C and N

Phytoplankton species composition in the epilimnion of the southwest basin did not respond to additions of C and N (Fig. 1), with the exception of 1977 and 1980. Chrysophytes domi-

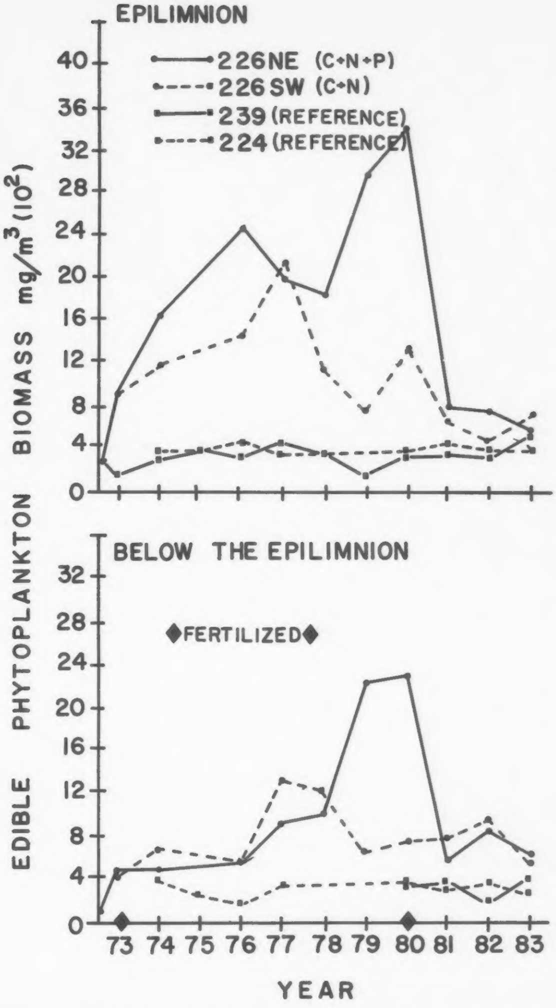

FIG. 3. Edible phytoplankton biomass (mg·m^{-3}) in the epilimnion and below the epilimnion for reference Lakes 224 and 239 and for Lake 226 northeast (C, N, and P additions) and Lake 226 southwest (C and N additions), 1973–83.

nated the assemblage constituting 75% of the standing crop (Table 2). Populations of diatoms, chlorophytes, cyanophytes, and dinoflagellates were also present. This assemblage was similar to that seen in our reference basins (Fig. 1). During 1977 and 1980 composition was erratic. Principal component analysis confirmed these observations (Fig. 2C).

In 1977 and 1980, chrysophytes declined in prominence as diatoms (*Rhizosolenia* and *Cyclotella*) and chlorophytes (*Oocystis* and *Spondylosium*) dominated in the spring and summer. Blooms of *Anabaena* in 1977 and *Oscillatoria* in 1980 occurred in late summer and lasted unitil ice cover in late November. The occurrence of these blooms can be related to leakage problems through the curtain separating the basins. A large vertical tear was detected in July 1977, and could have existed for as long as a month. There is strong evidence that mixing occurred between the northeast and southwest basins. Total phosphorus concentrations increased in the southwest basin (Fig. 5). The increased phosphorus caused a decrease in the TN:TP concentration ratio (Fig. 6) below 29:1, which coincides with the appearance of the cyanophyte *Anabaena*. Smith (1983) correlated increased cyanophycean abundance with a decline of the TN:TP concentration ratio below 29:1.

The late summer *Oscillatoria* bloom in 1980 is difficult to explain. In late August a small hole in the curtain was detected and repaired, and there was no evidence of mixing at this time (Fig. 5). Also, in late summer and fall the floats holding the surface collar of the curtain in place began falling off, possibly allowing some exchange of surface waters between the two basins but resulting in no detectable increase in total phosphorus. The *Oscillatoria* bloom occurred after nutrient additions had ceased and fall mixing had begun, and when the *Oscillatoria* population in the northeast basin was low. Its unusually high abundance (50% of total biomass) may be related to optimum combinations of light, temperature, and a change in the useable fraction of the nutrient pool.

There was a noticeable increase (2×) in the portion of the epilimnetic edible phytoplankton biomass in the southwest basin over the course of our additions (Fig. 3). This was due to increases in small flagellated chrysophytes and chlorophytes (Fig. 1). The two peaks which occurred coincided with the erratic species composition in 1977 and 1980. These peaks were due to large increases in abundance of chlorophytes. However, there was no increase in zooplankton biomass, but a shift from calanoids to herbivorous cladocerans was noted. There was also an increase in filter feeding rotifers (D. Malley, Freshwater Institute, personal communication).

During the course of C and N additions epilimnetic biomass in the southwest basin increased 3× over the 1971 Lake 226 value or 4× over the reference lakes mean for the same period (Table 3). Epilimnetic biomass averaged 3070 ± 1210 mg·m^{-3} from 1973 to 1980 (Table 3). A maximum increase of 6× (5700 mg·m^{-3}) occurred in 1977. Post-fertilization biomass decreased 0.6× to 1800 ± 550 mg·m^{-3} for 1981–83. This value is 2× higher than the pre-fertilized biomass in 1971 and is comparable to the increase seen in the (1981–83)/1971 ratio for the reference lakes. The increased average biomass in 1983 (5580 mg·m^{-3}) was due to a spring pulse of the diatom *Rhizosolenia* (Fig. 1 and 2, Table 3). Chemical monitoring ceased after 1982 and therefore we cannot determine if this diatom pulse was a response to nutrient changes.

As was the case in the reference lakes, the forest fire in 1979 had no detectable effect on the phytoplankton composition in either basin of Lake 226. However, the fire may have contributed to maintaining an elevated biomass even after nutrient additions ceased.

Lake 226 Northeast — Fertilized with C, N, and P

The epilimnetic phytoplankton assemblages in the northeast basin responded quickly to the additions of C, N, and P (Fig. 1). Species composition shifted from a sustained annual chrysophycean dominance in 1971 and spring 1973 to a codominance of chrysophytes, chlorophytes, and cyanophytes which continued for the eight years of fertilization. The most prominent change was an annual late summer cyanophycean bloom comprised of *Anabaena* (a nitrogen fixer) and *Oscillatoria* species (Table 2). Neither of these cyanophytes

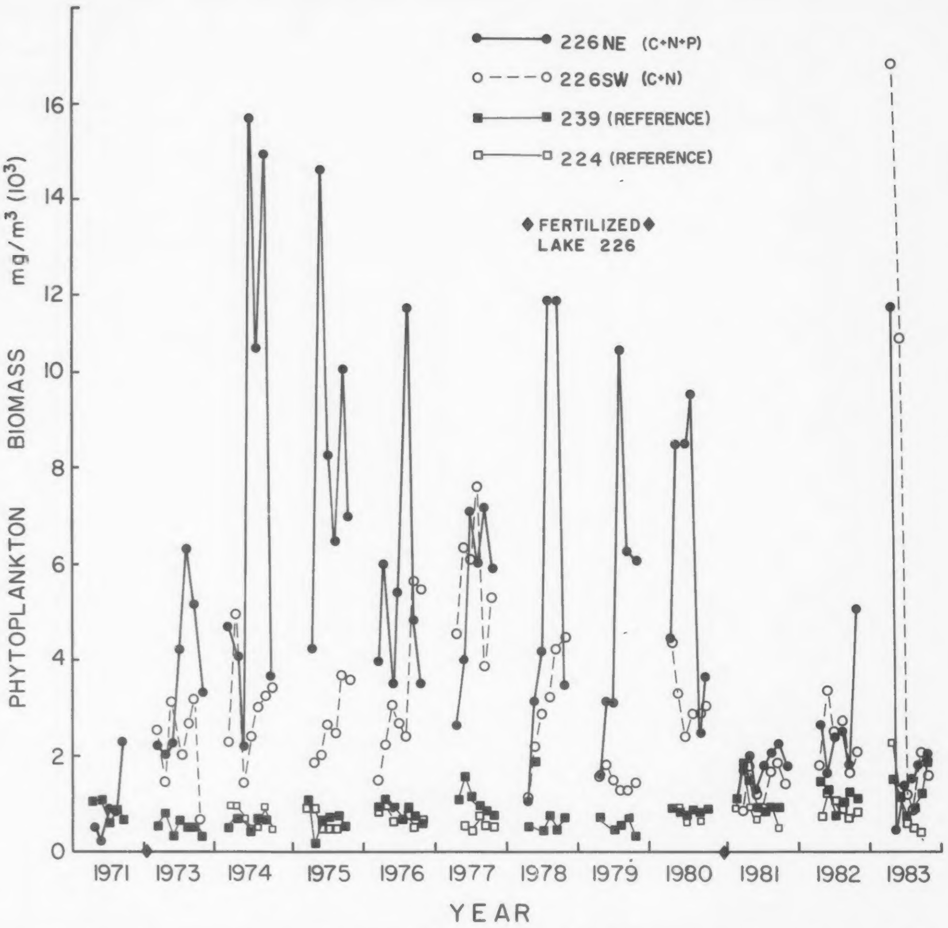

FIG. 4. Average monthly epilimnetic phytoplankton biomass ($\mathrm{mg \cdot m^{-3}}$) for reference Lakes 224 and 239 and for Lake 226 northeast (C, N, and P additions) and Lake 226 southwest (C and N additions) in 1971–83.

were found in the reference lakes. This shift to cyanophytes is evident in the principal component analysis (Fig. 2D). During fertilization years (1973–80, c–j), late summer samples fell outside of the reference lake cluster.

The appearance of cyanophytes in Lake 226 northeast can be directly attributed to the TN:TP concentration ratio which, during the fall and summer months of fertilization, remained below 29:1 (Fig. 5). The abundance of *Anabaena* can be attributed to the annual TN:TP loading ratio (by weight) which was less than 10:1 combined with ideal temperature conditions during summer. This supports Flett et al. (1980), who showed a strong correlation between the TN:TP loading ratio and the abundance of nitrogen fixing cyanophytes. When fertilization ceased the ratios increased above the critical levels and cyanophytes decreased in importance (Fig. 1, Table 2).

The dominant chlorophytes in the northeast basin were desmids, and they usually appeared in early summer prior to the cyanophycean bloom. Chrysophytes dominated in early spring after ice-out and in late fall and winter. Changes during fertilization were most pronounced in the epilimnion, but they were also observed to a lesser degree in the deeper strata (metalimnion and hypolimnion). However, cyanophytes never dominated the hypolimnion standing crop as they did in the epilimnion and metalimnion (Findlay and Kling 1975; Findlay 1978, 1981, 1983).

Because of changes in composition in the phytoplankton standing crop, there was a noticeable increase (10×) in the edible biomass (Fig. 2) which exceeded that seen in the southwest basin. This increase was due mainly to the appearance of large populations of desmids. Zooplankton showed similar responsed to those seen in the southwest basin (D. Malley, Freshwater Institute, personal communication).

Phytoplankton biomass estimates for the northeast basin also indicated an immediate response to nutrient enrichment. The average biomass value for 1973 to 1980 was 5920 ± 1330mg $\cdot \mathrm{m^{-3}}$ which was 6× the pre-fertilization value of 940 $\mathrm{mg \cdot m^{-3}}$ (1971). This is an 8-fold increase over the reference lakes and 2× over the southwest basin during the same period. As in the southwest basin, biomass declined (0.3×) after additions ceased and averaged 1650 ± 210 $\mathrm{mg \cdot m^{-3}}$ from 1981 to 1983

TABLE 3. Annual means of epilimnetic phytoplankton biomass, and loadings and epilimnetic cor
son. Lake 226 was fertilized from 1973 to 1980.

		Year									
	Lake	1971	1973	1974	1975	1976	1977	1978	1979	1980	1981
Mean epilimnetic phytoplankton biomass ($\mathrm{mg \cdot m^{-3}}$)	226NE		3670	7110	8160	5420	5350	5770	5510	6330	1900
		940									
	226SW		2350	2530	2970	3370	5700	2980	1530	3090	1490
	239	630	470	460	770	858	1040	750	560	820	1180
	224			720	610	750	660			750	930
Annual loading of total N (kg/ha)	226NE[b]	14.5	31.9	35.0	32.7	28.8	34.3	31.0	36.0	30.5	13.0
	226SW	14.1	29.5	30.8	29.5	26.9	30.8	28.2	33.3	28.2	11.0
	239	14.4	10.0	13.9	14.4	10.8	14.9	11.0	13.9	10.3	10.5
Annual loading of total P (kg/ha)	226NE[b]	1.1	4.2	4.1	3.8	3.7	3.9	3.7	3.7	3.7	0.29
	226SW	1.1	0.47	0.42	0.35	0.22	0.40	0.29	0.45	0.24	0.26
	239	1.0	0.51	0.56	0.51	0.30	0.47	0.39	0.42	0.30	0.27
Annual loading ratio TN:TP	226NE	13	7	7	7	7	7	7	8	8	42
	226SW	13	61	72	84	123	77	95	73	117	43
	239	14	19	25	28	36	32	29	33	35	39
Mean epilimnetic concentration of total N (μg/L)	226NE		410	510	520	580	450	760	610	600	370
	226SW		310	420	420	540	440	420	530	600	430
	239		246	330	280	350	340	290	320	330	280
Mean epilimnetic concentration of total P (μg/L)	226NE		16	18	25	21	21	34	23	25	10
	226SW		11	8	9	8	15	9	8	9	7
	239		6	8	6	7	7	7	6	6	7
Mean epilimnetic ratio TN:TP	226NE		26	28	21	28	21	22	27	24	37
	226SW		28	52	47	68	29	47	67	66	62
	239		41	41	46	51	48	41	53	55	41

[a] Annual mean computed by excluding early spring biomass value in 1983, which was g

[b] For 1973–82, N and P loadings to the northeast basin, due to flow from the southwest basi
of the southwest basin, and 0.5 of total outflow from the lake. There is no outflow data
from 1981 to 1982.

netic concentrations of total phosphorus and total nitrogen. All values are for the open water sea-

				Mean ±1 standard deviation					
					1981–1983		(1973–80)/	(1981–83)/	(1981–83)/
1981	1982	1983		1973–1980			1971	1973	1971
1900	1540	3230	(1520)[a]	5920±1330	220±890	(1650±210)[a]	6.3	0.3[a]	1.8[a]
1490	2430	5580	(1480)[a]	3070±1210	3170±2140	(1800±550)[a]	3.3	0.6[a]	1.9[a]
1180	1170	1250		720±200	1200±40		1.1	1.7	1.9
930	930	980		700±60	950±30			1.7	1.4
13.0	16.2	15.7		32.5±2.5	15.0±1.7				
11.0	11.9	12.8		29.7±2.0	11.9±0.9				
10.5	14.0	13.2		12.4±2.1	12.6±1.8				
0.29	0.41	0.51		3.9±0.2	0.40±0.11				
0.26	0.32	0.47		0.36±0.10	0.35±0.11				
0.27	0.44	0.47		0.43±0.10	0.39±0.11				
42	40	28		7.3±0.5	37±7.6				
43	37	27		88±22	36±8.1				
39	32	28		30±5.7	33±5.6				
370	370			560±110	370				
430	370			460±90	400±40				
280	310			310±40	300±20				
10	9			23±5.5	9.5±0.7				
7	9			9.6±2.4	8.0±1.4				
7	7			6.6±0.7	7.0				
37	41			25±3	39±3				
62	41			51±16	52±15				
41	44			47±6	43±2				

n was greatly inflated by *Rhizosolenia* bloom.

vest basin, were estimated from an open water season concentrations in the epilimnion
w data for 1971, and the 1983 value is estimated using average epilmnetic concentrations

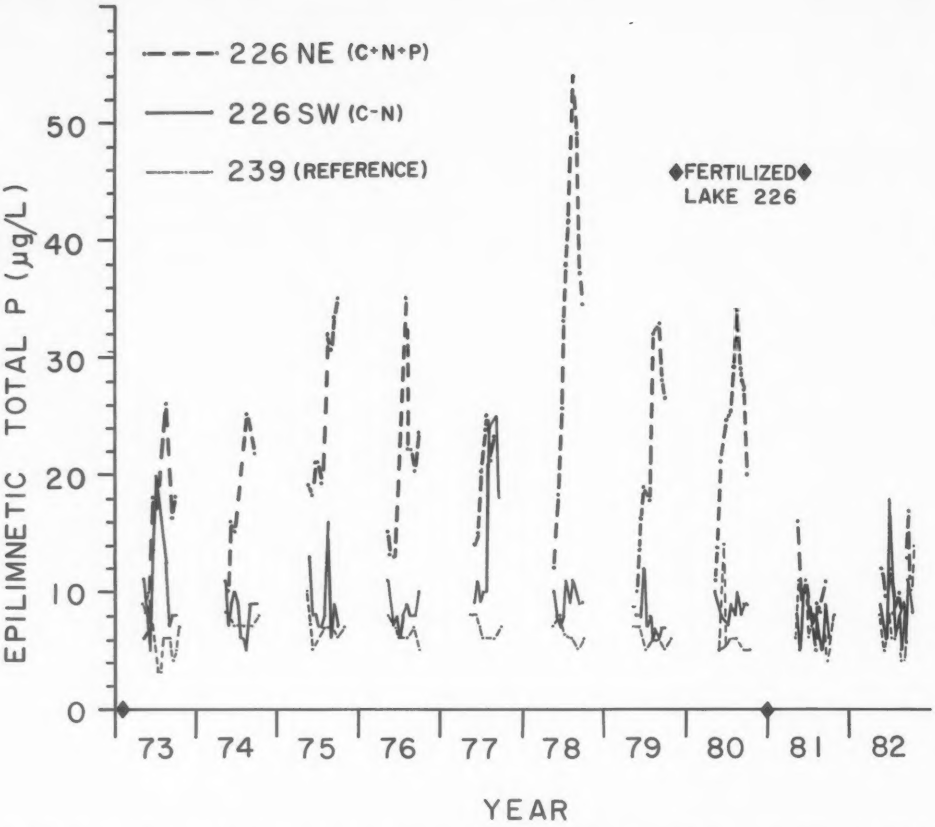

FIG. 5. Total epilimnetic phosphorus concentrations ($\mu g \cdot L^{-1}$) in Lake 226 northeast (C, N, and P additions) and Lake 226 southwest (C and N additions) and Lake 239 (reference), 1973–82.

which was twice the 1971 biomass value. The high mean (3230 $mg \cdot m^{-3}$) for 1983 was due to a spring pulse of *Rhizosolenia*.

Discussion

The northeast basin of Lake 226 received additions of carbon, nitrogen, and phosphorus at an N:P ratio of 5:1 (by weight). Dramatic shifts in phytoplankton species composition occurred immediately with chlorophytes dominating in early summer and cyanophycean blooms of *Anabaena* replacing these in late summer. *Anabaena* is a species capable of fixing atmospheric nitrogen. This domination and replacement sequence appeared in only one other lake receiving additions of nitrogen, and phosphorus. *Aphanizomenon*, another N_2fixer, dominated in Lake 227 after the ratio was decreased to 5:1 from 15:1 by weight in 1975 (Schindler 1977). The appearance of these species in both lakes receiving nitrogen and phosphorus at a ratio below 10:1 supports the findings of Flett et al. (1980). It is apparent these species compensate for a nitrogen deficiency in a phosphorus-rich environment.

In addition to Lake 226, several other whole lake nutrient experiments have been conducted to examine the roles of carbon, nitrogen, and phosphorus in stimulating phytoplankton responses. Lake 227 (1969–74) in the Experimental Lakes Area (Schindler 1974, 1975, 1977) and Lake Magnusjaure (1974–75), Hymenjaure (1975), and Gunillajaure (1978–79) in Sweden (Holmgren 1983) all received epilimnetic additions of nitrogen and phosphorus at a ratio of ≃15:1 (by weight). These lakes all responded similarily to the northeast basin of Lake 226, with chlorophytes or cyanophytes eventually dominating the phytoplankton assemblage. However, no nitrogen fixing cyanophycean blooms occurred in any of these lakes. Lake 304 (1972–73) received areal loading of nitrogen and phosphorus similar to Lake 227 and the Swedish lakes, but also received carbon (Schindler 1975). Similar responses to those seen in Lake 227 and the Swedish lakes occurred, indicating there was no additional effect from carbon.

In addition to changes in species composition, biomass in the northeast basin exhibited an average annual increase of 8 × over reference systems with a seasonal maximum of 21 600 $mg \cdot m^{-3}$ in 1974. Holmgren recorded average biomass increases of 20 × over background levels in Lake Gunillajaure. The increased biomass was directly related to the additions of nitrogen and phosphorus. During recovery years in both the Lake 304 (1973–74) and the Lake 226 (1981–83) experiments there was an immediate decrease in epilimnetic phytoplankton biomass.

The southwest basin was intended as a control for the northeast basin, and it was hypothesized the phytoplankton com-

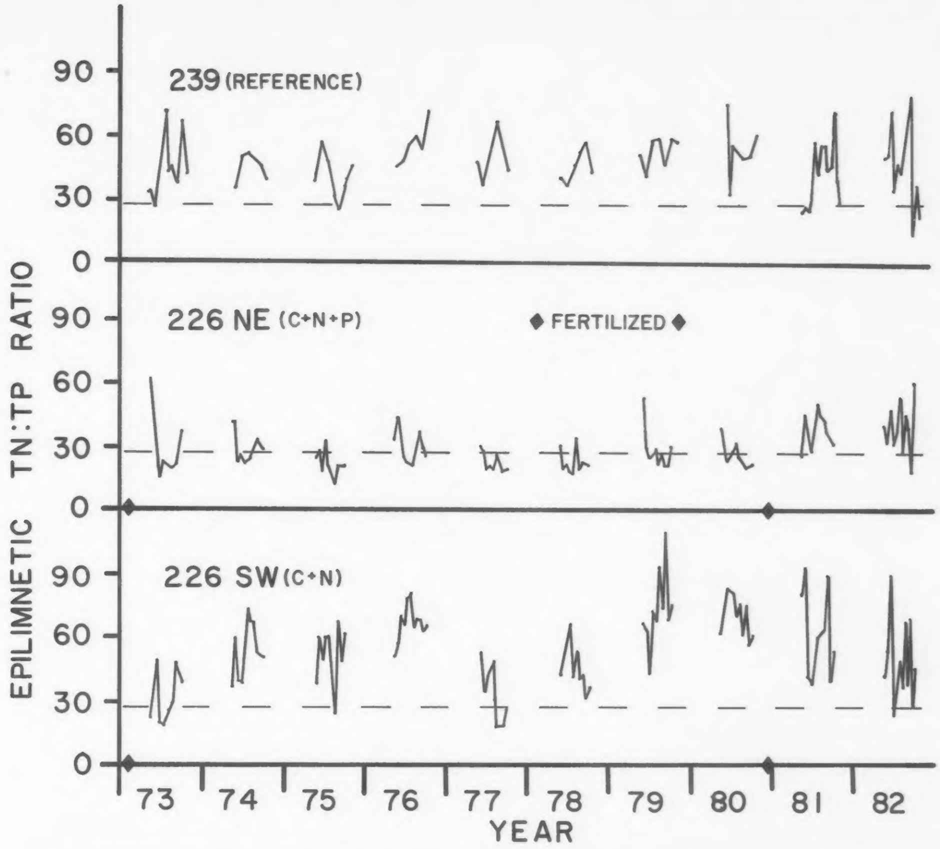

FIG. 6. Epilimnetic total nitrogen:total phosphorus ratio (by concentration) for the northeast (C, N, and P additions) and southwest (C and N additions) basins of Lake 226 and for Lake 239 (reference), 1973–82.

munity would not be stimulated by addition of carbon and nitrogen. But as seen in Fig. 4 and Table 3, there was a detectable increase in epilimnetic phytoplankton biomass (3×) when compared to that before fertilization. This increase could have been a response to several factors.

It is unlikely that responses seen in the southwest basin can be attributed to partitioning the lake into two basins (curtain effect). The natural flow is from south to north and the whole-lake water residence time averaged 6.5 yr. After installation of the curtain, water residence time in the southwest basin remained approximately the same (6.9 yr), but it decreased to 3.3 yr in the northeast basin. Therefore, partitioning could affect only the northeast basin because it was being diluted by low phosphorus water from the southwest basin combined with an increased flushing rate. These factors imply that we might have expected even higher biomass increases in the northeast basin.

The increased phytoplankton biomass in the southwest basin was significantly higher than natural increases observed in the reference lakes (239 and 224). During the study period, phytoplankton biomass in Lake 239 remained relatively constant from 1971 to 1980 and increased 2× in 1981 to 1983 (Table 3). Phytoplankton biomass increased 3× in the southwest basin of Lake 226 from 1971 to 1980 and then decreased in 1981 to 1983 after additions ceased. Though post fertilization biomass in the southwest basin remained 2× higher than the prefertilization estimates in 1971, this increase was similar to that in the reference lakes for the same period.

A more plausible explanation of the increase in biomass could be that it was due to additions of nitrogen. The literature on this point is contradictory. Holmgren (1983) fertilized Lake Magnusjuare (1974) with a similar loading of nitrogen and saw a 7× increase in biomass. However, Lake 304 (E.L.A.) was fertilized with both nitrogen (ammonia chloride) and phosphorus in 1971 and 1972, and with nitrogen alone from 1973 to 1974. In 1973 biomass declined to values similar to prefertilization and there was no response to the continued additions of nitrogen (Schindler 1975).

Another possible explanation for the increase in phytoplankton biomass is leakage of water between the two basins. Although the curtain was weighted to the lake bottom it was not sealed to it and some mixing could occur. Also, large leaks occasionally occurred, and sloppage over the surface collar was observed. Figure 5 and Table 3 indicate there was no obvious increase in epilimnetic phosphorus in the south basin except in 1977. However, a 10% increase might not be detected by our analytical methods, or it might be obscured by seasonal variation. This increase in epilimnetic phosphorus may have been sufficient to stimulate an algal response, and could account for the increases in small chrysophytes and chlorophytes without massive blooms of cyanophytes.

Due to these complicating factors, it is difficult to determine the significance of the phytoplankton community response to the nitrogen additions in the southwest basin. However, phosphorus additions in the northeast basin of Lake 226 significantly altered the phytoplankton species composition and the proportion of edible biomass, and increased the total biomass as compared to the southwest basin which received only nitrogen and carbon.

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Phytoplankton Productivity Changes in a Small, Double-Basin Lake in Response to Termination of Experimental Fertilization

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One basin of a small, double-basin lake was fertilized with carbon, nitrogen, and phosphorus for eight years, and then fertilization was stopped. The other basin was fertilized simultaneously with equivalent amounts of carbon and nitrogen only. Phytoplankton primary production was monitored using an incubator–numerical model technique. Production increased dramatically in the basin receiving artificial additions of C, N, and P. The increase was particularly large in the epilimnion where Cyanophyte blooms occurred during each year of fertilization and production rates averaged 2 to 10 times higher than in nearby, unfertilized reference lakes. Phosphorus, not nitrogen or carbon, was the critical nutrient. The productivity of the other basin also increased, but to a lesser degree and no Cyanophyte blooms were observed in this basin. When all fertilization was terminated, production in both basins immediately decreased. No more surface blooms were observed in either basin. Within 3 yr, the production had dropped to levels typical of reference lakes.

Un bassin d'un petit lac à double bassin a été fertilisé pendant huit ans par des additions de carbone, d'azote et de phosphore. Le second bassin était aussi fertilisé simultanément avec des quantités équivalentes de carbone et d'azote. La production phytoplanctonique primaire a été contrôlée par technique incubateur-modèle numérique. La production s'est accrue de façon extrêmement importante dans le bassin fertilisé par C, N et P. L'augmentation était particulièrement importante dans l'épilimnion où il y avait croissance abondante de cyanophytes à chaque année; les taux de production étaient en moyenne de 2 à 10 fois supérieurs à ceux des lacs témoins avoisinants non fertilisés. La matière nutritive critique était le phosphore et non l'azote ou le carbone. La productivité de l'autre bassin s'est aussi accrue, mais à un moindre degré, et il n'y avait pas surcroissance des cyanophytes. La production des deux bassins a immédiatement décliné dès que l'on a mis fin à la fertilisation. Il n'y avait plus aucune croissance d'algues en surface. En moins de 3 ans, la production était revenue au niveau de celle des lacs témoins.

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During the early 1970's, considerable controversy surrounded the issue of which nutrient was the key to the control of eutrophication. While most research pointed to phosphorus (Vollenweider 1968; Edmondson 1970; Vallentyne 1970; Schindler 1971), others argued that carbon was the most important nutrient (Kuentzel 1969; Lange 1970, 1971). To solve this controversy, a whole-lake experiment was undertaken (Schindler 1974; Schindler and Fee 1974). The essence of this experiment was to add equal quantities of carbon and nitrogen to two basins of a single lake, but to add phosphorus to only one basin.

Lake 226 (L226) in the Experimental Lakes Area (ELA), northwestern Ontario, is a small double-basin lake. The two basins, northeast (NE) and southwest (SW), are almost equal in surface area (8.3 and 7.8 ha) and similar in mean depth (5.7 and 6.3 m). Maximum depths are 14.7 and 11.6 m, respectively. The basins are connected by a channel about 50 m wide and 3 m deep. At the beginning of the experiment, this channel was closed with a reinforced vinyl "sea curtain" which prevented exchange of water between basins. L226 is a headwater lake with a small drainage basin and the natural flow, from the southwest to the northeast basin, was small (range = 0.000 to 0.045 $m^3 \cdot s^{-1}$; mean = 0.005 $m^3 \cdot s^{-1}$) (Beaty 1981, 1984).

Following installation of the curtain in May, 1973, the two basins received weekly additions of fertilizer during the ice-free periods (May through October) for eight consecutive years (Schindler 1975; Cruikshank 1984). Both basins received equivalent additions of carbon and nitrogen. Phosphorus also was added to the northeast basin while the southwest basin received no experimental phosphorus additions.

The general responses of the L226 basins to the first four years of fertilization have been reported previously (Schindler and Fee 1974; Schindler 1975, 1977, 1980; Fee 1979, 1980). Each summer, a surface bloom of blue-green algae (Cyanophyta) developed in the northeast basin, the basin receiving phosphorus additions. The blooms usually developed in mid- to late August and persisted for several weeks, with both *Anabaena*, a nitrogen fixer, and *Oscillatoria* species typically present (Findlay and Kling 1975; Findlay 1978). These blooms varied in magnitude from year to year, but they always occurred and were always visually apparent (For example, see Schindler 1974). Primary production in both basins increased during the first years of fertilization, but the production increase was much greater in the basin which received experimental additions of phosphorus (Fee 1979, 1980). These differences in the algal responses of the two basins were attributed to the effects of the phosphorus added to the northeast basin (Schindler 1975, 1977, 1980).

All fertilizer additions were terminated at the end of 1980, but the curtain was maintained until July 1984, thus providing an opportunity to observe the "recovery" of the fertilized basins. This paper will summarize the phytoplankton productivity responses of both basins to 8 yr of fertilization, with emphasis on the last 4 yr which were previously unreported. In addition, it will detail the recovery from fertilization observed in the first 3 yr after fertilization was stopped.

Methods

The methods of fertilization have been documented by Cruikshank (1984). Some variations in methods occurred over the years: during 1973 and most of 1974, fertilizer was distributed once per week in the epilimnetic waters of both basins by mixing from the prop-wash of a boat; in late 1974, a change to semi-continuous point source addition was made (trickle-feed from barrels on shore). Then the location of these point sources were changed: initially, the barrels were located within 15 m of the curtain separating the basins, but to minimize the interbasin transfer of phosphorus resulting from leakage around the curtain, the barrels were moved in late 1977 to small islands more centrally located in each basin.

Sodium nitrate ($NaNO_3$) was used as the nitrogen source, sucrose was the carbon source, and phosphoric acid (H_3PO_4) was the phosphorus source. Annual addition rates were 3.16 $g \cdot m^{-2}$ for N and 6.05 $g \cdot m^{-2}$ for C in both basins, and 0.59 $g \cdot m^{-2}$ for P in the NE basin (Schindler 1975). The C:N:P mass ratio added to L226NE was 10:5:1. This N:P ratio of 5 was chosen to encourage the development of nitrogen-fixing, blue-green algae (Schindler 1977). Total N and total P concentrations were determined as described in Stainton et al. (1977).

While the nylon-reinforced, vinyl curtain was generally effective at preventing exchange between the two basins, there was some transfer of phosphorus from the NE to the SW basin. On several occasions (July and August, 1977; September, 1978; July, 1980), significant leaks went undetected for periods of up to 4 wk. During these periods, fish tagged in one basin were able to move to the other basin (Mills and Chalanchuk 1987). There was also a significant exchange of water and nutrients on some of these occasions; most notably in August, 1977, when total P concentrations in L226SW suddenly increased and remained higher than in the northeast basin for several weeks (Findlay and Kasian 1987; ELA staff, unpublished data).

From 1973 to 1983, phytoplankton primary production for L226 was estimated using an incubator–numerical model (Fee 1973). Water samples were collected at regular time intervals from the epilimnion in each basin and returned to the laboratory where carbon uptake rates versus photosynthetically active radiation (PAR) were measured using radiocarbon tracer techniques in artificial light incubators. Samples were incubated at in situ temperatures for periods of 2–4 h. Post incubation processing was by acidification and bubbling (Schindler et al. 1972), and liquid scintillation spectrometry. Surface solar radiation was measured continually during the ice-free periods using a quantum sensor (Li-Cor 190S). Similar sensors (Li-Cor 192S) were used to measure vertical attenuation of PAR within the water columns of both basins. Shearer et al. (1985, 1987) describe field and laboratory methods.

Although the primary purpose of this study was to compare productivity responses in the two basins of L226, several nearby, non-fertilized reference lakes were also monitored using methods identical to those employed for L226. Lakes 222, 224, 239, 240, 302 South, 382, and 383 were among the untreated lakes for which phytoplankton production was measured, although only L239 was sampled in each year of the L226 study.

From 1976 through 1983, chlorophyll concentrations were measured from aliquots of the same samples used for productivity incubations. The algal cells were trapped on glass fibre filters (Whatman GF/C) and, until 1981, the chlorophyll was extracted by grinding these filters in 90% acetone for 1 min and filtering the resulting slurry through a clean filter (Stainton et al. 1977). In subsequent years, chlorophyll was extracted by allowing the filters to stand overnight in 95% methanol at 4°C (M. P. Stainton, Freshwater Institute, Winnipeg, unpublished data). In both methods, the chlorophyll concentrations were determined by assay in a calibrated fluorometer (Turner 111).

Techniques were refined and equipment improved throughout the 11-yr study period. Integrated sampling (Fee 1976) replaced spot sampling in 1974 and vacuum bubbling (Wessels and Birnbaum 1979) replaced Allihn tubes in 1981. A more efficient liquid scintillation spectrometer was purchased in 1979 and an improved incubator design (Shearer et al. 1985) was introduced in 1982. Scalar quantum sensors (1977) and a new liquid scintillation fluor (1981) helped to improve our measurements. Whenever such changes occurred, intercalibrations were performed to determine if the old and new methods were significantly different. Corrections for such differences are incorporated in the long-term comparisons of data.

The numerical model used for estimating production integrated over time and depth ("integral production") evolved as various assumptions were tested and either accepted or rejected (Fee 1977, 1984). The implications of diurnal variation, surface inhibition, and light spectral quality were considered (Fee 1975, 1979) and provisions for these effects were included in early versions of the model (Fee 1977). Later research (Bower 1981; Shearer et al. 1985) showed that these effects were not significant and corrections for these factors no longer were made.

Method changes complicate comparisons among different years. Fee (1980) and Shearer et al. (1985) have discussed these problems in more detail. For the purposes of this paper, the integral values calculated by the model used in 1980 have been selected as a standard for all calculations. This model was used to recalculate all data from 1976 through 1980 (Fee et al. 1982) and to estimate integral rates for 1981 and 1982. a revised model (Fee 1984) was used for the 1983 data after it was checked extensively against the previous model and was found to give similar results (± 10%). Data for the years 1973 through 1975 were not recalculated using the 1980 or 1983 models because the input data were not compatible with these newer models. However, the production estimates for those years were adjusted upward by 16% in the northeast basin and by 24% in the southwest basin to match the change observed in the results for subsequent years when they were recalculated. The new incubator design introduced in 1982 produced L226

TABLE 1. Summary of phytoplankton productivity estimates and relative
ice-free seasons. Daily values are seasonal means, either for the entire eup
maximum uptake rates measured in the incubator for an epilimnion samp
irradiance (I_o) measured at 3.0 m each year. Fertilization of the two basi
nutrients, ceased at the end of 1980

		Phytoplankton carbon uptake estim				
		Ice-free total	Eup mean daily		Epi mean daily	
Year	Basin	(g $C \cdot m^{-2}$)	mg $C \cdot m^{-2}$	NE/SW	mg $C \cdot m^{-2}$	N
1973	NE	66	358		299	
	SW	57	309	1.16	225	
1974	NE	87	480		422	
	SW	49	273	1.76	200	
1975	NE	69	361		304	
	SW	55	288	1.25	185	
1976	NE	91	524		464	
	SW	43	247	2.12	188	
1977	NE	101	497		410	
	SW	79	388	1.28	295	
1978	NE	148	775		686	
	SW	81	423	1.83	288	
1979	NE	149	803		707	
	SW	64	342	2.35	244	
1980	NE	145	786		695	
	SW	94	507	1.55	385	
73–80	NE	107	573		498	
	SW	65	347	1.65	251	
1981	NE	74	350		272	
	SW	63	299	1.17	204	
1982	NE	65	338		254	
	SW	56	289	1.17	194	
1983	NE	41	208		158	
	SW	28	143	1.45	103	
81–83	NE	60	299		228	
	SW	49	244	1.23	167	

relative photosynthetically active radiation (PAR) at 3 m for Lake 226 during the
ntire euphotic zone (Eup) or for the epilimnion (Epi) only. Hourly values are the
ion sample. PAR data represent the mean and minimum percentages of surface
two basins, which differed in that the NE basin received P in addition to other

ke estimates				Relative PAR at 3 m			
pi mean daily		Hourly maximum		Mean		Minimum	
m^{-2}	NE/SW	mg $C \cdot m^{-3}$	NE/SW	$\%I_o$	NE/SW	$\%I_o$	NE/SW
		50.5		11.5		4.6	
	1.33	13.6	3.71	15.4	0.75	6.4	0.72
		44.3		6.2		0.3	
	2.11	9.8	4.52	12.6	0.49	9.0	0.03
		28.3		8.4		2.1	
	1.65	10.5	2.70	11.0	0.76	7.3	0.28
		54.0		10.4		6.7	
	2.47	9.5	5.68	12.1	0.86	8.1	0.82
		23.1		7.2		2.3	
	1.39	19.5	1.18	9.1	0.79	3.8	0.61
		95.8		4.3		0.2	
	2.38	11.2	8.55	9.9	0.43	4.2	0.04
		50.2		7.3		2.8	
	2.90	11.4	4.40	13.6	0.54	4.0	0.70
		61.1		6.2		2.4	
	1.81	26.1	2.34	9.7	0.64	6.5	0.36
		50.9		7.7		2.6	
	1.98	14.0	3.65	11.7	0.66	6.1	0.43
		13.9		13.9		8.0	
	1.33	12.2	1.14	13.8	1.01	8.4	0.96
		14.0		10.9		4.3	
	1.31	11.6	1.21	10.8	1.01	4.6	0.93
		8.7		11.2		6.2	
	1.53	6.5	1.34	11.8	0.95	6.4	0.97
		12.2		12.0		6.2	
	1.37	10.1	1.21	12.1	0.99	6.4	0.96

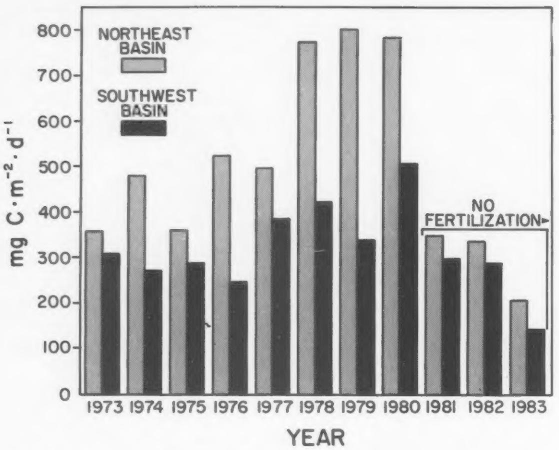

FIG. 1. Annual ice-free mean integral daily phytoplankton production rates (mg $C \cdot m^{-2} \cdot d^{-1}$) for the euphotic zone of Lake 226, 1973 through 1983.

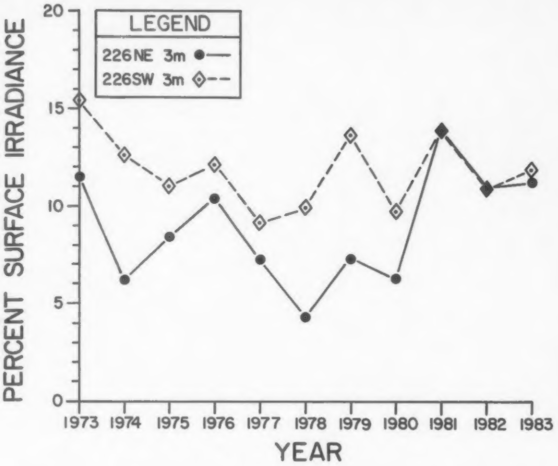

FIG. 2 Mean percentage of surface photosynthetically active radiation penetrating to 3.0 m in each basin of Lake 226 during each ice-free season, 1973 through 1983.

integral production estimates which averaged 20% lower than did estimates from the previous incubator. For making comparisons, we have adjusted the 1982 and 1983 results upward by 20%.

It is not possible to put precise confidence limits on our integral production data. Incubator carbon uptake rates normally are replicable to within 5%. It is more difficult to determine the magnitude of errors introduced by using linear interpolation between carbon uptake and vertical light attenuation data measured at approximately 2-wk intervals to estimate total production for the ice-free season. Tests carried out in L226 in 1978, in conjunction with whole-lake radiocarbon addition (Bower 1981), showed that independent production estimates for a given day, based on samples obtained and incubated on different days of the same week, were usually within 20% of each other. Also, both basins of L226 were sampled on the same days and any biases inherent in the techniques, including weather variations and length of period between sampling dates, should have been the same for both basins.

Phytoplankton production is usually expressed in this paper as daily mean carbon uptake under simulated cloudless skies (Table 1). This eliminates the biases which would otherwise result from annual differences in the lengths of the ice-free period (173 days to 210 days) during which measurements were made, and from annual differences in cloud cover. At the ELA, these cloudless estimates have averaged 1.42 ± 0.04 times those based on actual weather. To facilitate comparisons with other studies, annual ice-free euphotic zone totals also are listed in Table 1.

Results

In L226NE, the basin fertilized with carbon, nitrogen, and phosphorus, phytoplankton production increased during the eight years of treatment (Table 1) and was consistently higher than in the southwest basin to which was added only carbon and nitrogen. Production rates per unit area for the entire euphotic zone ($I_z > 0.5\%$ of I_o) of L226NE during the final 3 yr of fertilization averaged approximately twice those rates measured during the first 3 yr of the study (Fig. 1). Blooms of Cyanophyta (Findlay 1981, 1983) continued to form during each summer that the nutrients were added. Epilimnetic light attenuation greatly increased during each August and September, such that, on at least two occasions (1974, 1979), less than 0.5% of the surface PAR was able to penetrate to a depth of 3.0 m (Table 1). Maximum epilimnetic production rates (23–96 mg $C \cdot m^{-3} \cdot h^{-1}$) (Table 1) during these blooms were 5 to 10 times higher than in untreated ELA lakes (see, for example, DeClercq and Shearer 1979) and epilimnetic production contributed from 83% to 89% of the euphotic zone totals for the northeast basin during the fertilization years.

In the southwest basin of L226, which received no phosphorus additions, production increased during the last 4 yr of fertilization (Fig. 1), but the increase and the rates (Table 1) were similar in magnitude to those observed in some untreated ELA reference basins during the same period (Shearer et al. 1987). No extremes of production were measured in the epilimnetic waters of the southwest basin and irradiance levels in the deeper waters were consistently higher than in the northeast basin. On average, the percentage of PAR reaching 3.0 m was 150% greater than in the northeast basin during the fertilization years, and more than 230% greater during bloom periods (Table 1, Fig. 2). This lower light attenuation resulted in deeper mixed layers in the southwest basin, but it also permitted a greater proportion of the total production to occur in algal populations deep in the water column. Epilimnetic production never exceeded 76% of total production in the southwest basin (Table 1). In some years (1976, 1978, 1979), less than one third as much phytoplankton production occurred in the top metre of the L226SW water column as in the top metre of L226NE. For the 1973–80 period, total production per unit area in the southwest basin averaged only 61% ± 16% of that in the northeast basin, while epilimnetic production per unit area averaged 51% ± 14% of the northeast value (Table 1).

Production in L226NE decreased immediately when fertilization was stopped (Table 1, Fig. 1). Mean daily production in the first non-treatment year (1981) was only 44% of the mean of the previous 3 yr for the euphotic zone and 39% for

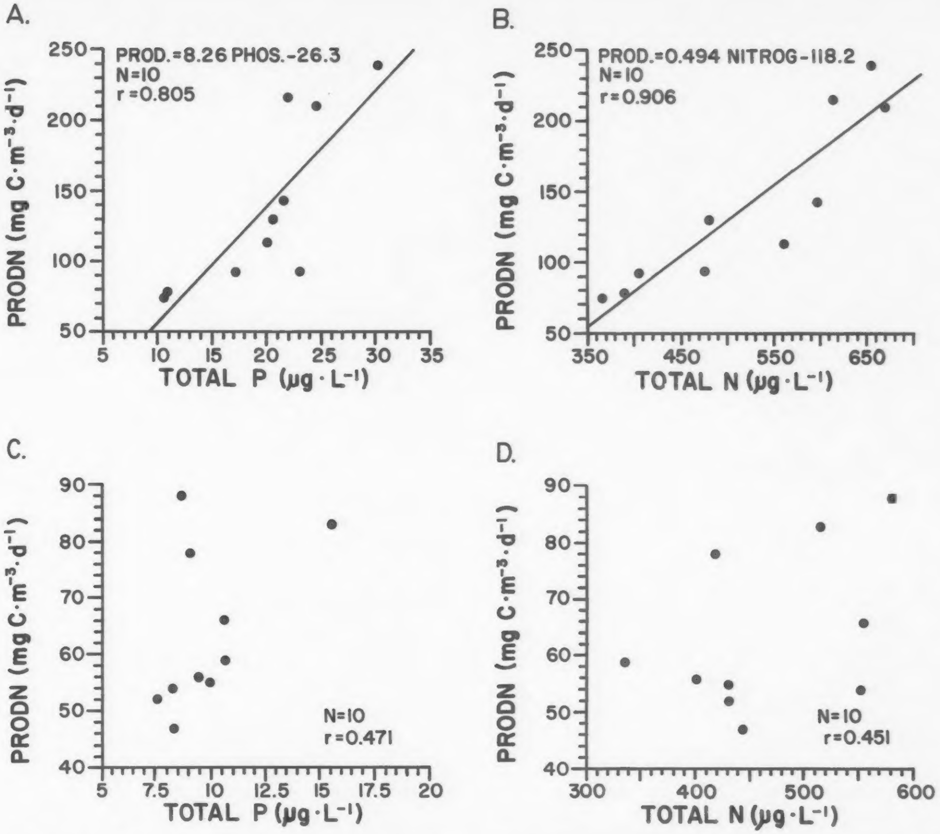

FIG. 3 Relation of mean epilimnetic production rates to mean epilimnetic concentrations of total phosphorus (A and C) and total nitrogen (B and D) for L226NE (A and B) and L226SW (C and D), 1973 through 1982.

the epilimnion. This trend continued during the following 2 yr as production rates decreased well below any previously measured in the basin. No surface blooms occurred after fertilization was stopped. Attenuation of PAR decreased (Fig. 2) and metalimnetic production, as a proportion of total production, increased.

Production in the southwest basin also decreased after treatment ceased (Table 1, Fig. 1). The 1981 mean daily production was 71% of the 1978−80 mean for the euphotic zone and 67% for the epilimnion. By the third year of recovery, rates were much lower than any measured in previous years. The attenuation of PAR in the epilimnion was unchanged from the treatment years and almost identical to that in the northeast basin during the post-treatment years (Table 1, Fig. 2). During the three year recovery period, total production per unit area in L226SW averaged 81% ± 11% that of L226NE; epilimnetic production averaged 73% ± 6% of the northeast basin value (Table 1).

Mean epilimnetic production rates in the NE basin for 1973 through 1982 were more closely correlated with ice-free mean, epilimnetic, total phosphorus concentrations ($r = 0.805$, $n = 10$, $P < 0.01$) than were analogous values from the SW basin ($r = 0.471$, $n = 10$, $P > 0.10$) (Fig. 3). Mean epilimnetic production rates in the NE basin were even more highly correlated with ice-free mean, total nitrogen concentrations ($r = 0.906$, $n = 10$, $P < 0.001$). There was no significant correlation between phytoplankton production and nitrogen concentration in the epilimnion of the SW basin during this 10-yr period ($r = 0.451$, $n = 10$, $P > 0.10$) (Fig. 3). No nutrient concentrations were measured in 1983.

Mean epilimnetic production rates for 1976 through 1983 were highly correlated with mean annual epilimnetic chlorophyll concentrations for L226NE ($r = 0.968$, $n = 8$, $P << 0.001$,), but less strongly correlated with mean epilimnetic chlorophyll for L226SW ($r = 0.779$, $n = 8$, $P < 0.02$) (Fig. 4). No directly comparable chlorophyll data were available for the 1973−75 period. Mean epilimnetic production was negatively correlated with mean epilimnetic transparency in the NE basin ($r = -0.826$, $n = 11$, $P < 0.01$) but not in the SW basin ($r = -0.557$, $n = 11$, $P > 0.10$).

Although the primary purpose of the experiment was to compare the responses of the two L226 basins, their productivity relative to reference lakes was also examined. Fee (1979) demonstrated a relationship between the ratio of the area of the epilimnetic sediments (A_e) to the volume of the epilimnion (V_e) and the annual epilimnetic primary production (Epi Prod). An updated calculation, using 49 lake-years of data from 1973−83 for stratified reference basins at the ELA (Fig. 5), illustrates a highly significant positive correlation between Epi Prod and A_e:V_e ($r = 0.79$, $P < 0.001$). When superimposed on these

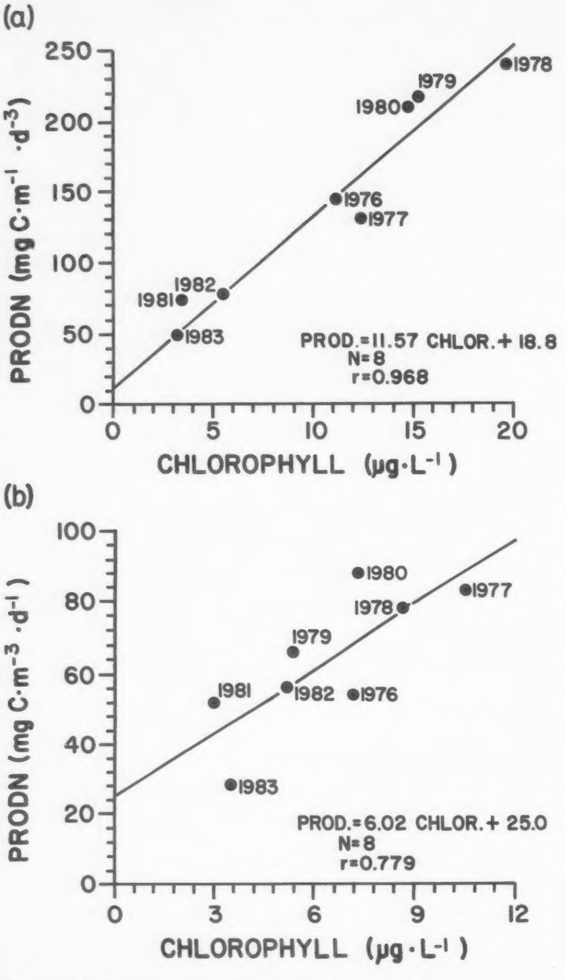

FIG. 4. Relation of mean epilimnetic production rates and mean epilimnetic chlorophyll concentrations for (a) L226NE and (b) L226SW, 1976 through 1983.

data for the reference lakes, the values for the NE basin during the fertilization years all lie well above the 95% confidence limits for the reference basins. Seven of the eight values for the SW basin during these same years are also above the 95% limits, although to a lesser degree. The 1981 and 1982 values for both basins were much closer to the expected values for reference basins. By 1983, epilimnetic production in both basins of L226 was well within the limits expected for ELA reference basins of similar morphometry.

Discussion

The production estimates for L226 during the last 4 yr of fertilization supported the conclusion (Schindler and Fee 1974; Schindler 1975, 1977) that phosphorus is the key nutrient controlling eutrophication. Production was enhanced more in the northeast basin, the basin which received experimental additions of phosphorus, than in the southwest basin which received only carbon and nitrogen. Cyanophyte blooms developed in L226NE every year that phosphorus was added (Findlay 1981, 1983).

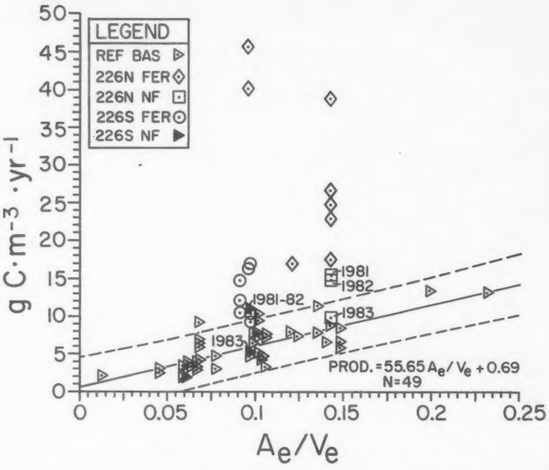

FIG. 5. Relation of epilimnetic production to A_e:V_e (see text) in stratified ELA lakes, 1973 through 1983. The regression was calculated from 49 lake-years of data for untreated ELA reference basins (REF BAS). The dashed lines are the 95% confidence limits. The L226 values for the same years are superimposed (FER = fertilized, NF = not fertilized).

The ratio of N to P added to the lake also was critical in determining the algal response. The high correlation between total nitrogen and phytoplankton production in the epilimnion of the NE basin (Fig. 3) indicates that nitrogen often became limiting in this basin during the fertilization period. The N to P addition mass ratio of 5:1 created conditions whereby the phytoplankton in this basin often had a surplus of phosphorus relative to the readily available nitrogen. The appearance each summer of nitrogen-fixing *Anabaena* blooms (Findlay and Kasian 1987) was a natural response to this nitrogen limitation.

Much of the apparent productivity increase in the southwest basin can be attributed to leakage of phosphorus-rich water from the northeast basin past the curtain. This may partially explain why the productivity rates in the two basins during 1977 were similar, and why southwest basin production was elevated in 1980. Some blue-green algae did appear in the summer plankton of the southwest basin, but only in 1977 and 1980 did they ever dominate or reach bloom proportions (Findlay and Kasian 1987). Their presence was used as an indication that the sea curtain required repairs. In 1977, the nutrient source barrels were still located close to the curtain and in August of this year a leak occurred which permitted considerable mixing between the basins. Phosphorus concentrations in the southwest basin more than doubled during this period (ELA staff, unpublished data). As a result,nitrogen became limiting and a bloom of nitrogen-fixing *Anabaena* occurred (Findlay and Kasian 1987). After the 1977 leakage, the nutrient sources were moved away from the curtain and the probability of concentrated phosphorus transfer was reduced. The curtain leakage in 1980, although less obvious, was prolonged. By then, the surface flotation on the curtain separating the basins had begun to deteriorate. There was an increased transfer of water between basins because of waves slopping over the surface floats, perhaps assisted by northeast winds. Although no increases in phosphorus concentration were detected in the southwest basin, we are certain that some phosphorus-rich water from the northeast basin was being transferred to the southwest basin over the course of the summer. However, nitrogen apparently did not become limiting and *Oscillatoria*, not *Anabaena*, formed a bloom in L226SW in 1980. The fact that epilimnetic production in L226SW was above the expected range for untreated lakes in 7 of 8 treatment years (Fig. 5) suggests either that the added N had some positive effect on production or that some leakage of P past the curtain occurred in most years.

Of particular importance for lake management is the sudden and significant decline in production which followed the cessation of artificial nutrient enrichment. The production in the northeast basin in 1981, the first year of recovery, was less than half that measured in the same basin in each of the last 3 treatment years. This decrease occurred in a year when many untreated lakes in the ELA were showing productivity increases (Shearer et al. 1987). The productivity of the southwest basin also decreased during this period, albeit to a lesser degree (Table 1). For the first time in 9 yr, no Cyanophyte blooms occurred in L226NE. This is extremely significant, because these surface blooms are a major factor in the determination of the water quality and the general aesthetics of lakes. Within 1 yr, the major differences between the two basins caused by phosphorus additions (productivity enhancement, Cyanophyte surface blooms and lower transparency) had virtually disappeared (Table 1; Fig. 1 and 2).

Such a large and rapid decrease in productivity after phosphorus cutoff implies that the efficiency of phosphorus removal from L226NE waters each year was very high. Schindler et al. (1978) noted that equilibria between ELA lakes and their phosphorus input are established quickly and that most of the phosphorus is rapidly sedimented. Phosphorus concentrations are highly dependent on phosphorus input. During the early years of phosphorus addition, nitrogen levels in the northeast basin were low relative to algal requirements. Nitrogen fixation, which was occurring during the fertilization period (Flett et al. 1980; Graham et al. 1980), was probably slowly reducing this nitrogen deficiency. Once the nutrient input was cut off, both basins readjusted quickly to the new phosphorus levels.

Many other studies (Edmondson 1970; Michalski and Conroy 1973; Schindler 1974; Larsen et al. 1975; Michalski et al. 1975) have shown, by demonstrating major reductions in chlorophyll concentrations, that significant recovery from cultural eutrophication can occur rapidly following nutrient reduction. Few included data on phytoplankton production. The significant correlations in L226NE (Fig. 4a) and L226SW (Fig. 4b) between annual mean epilimnetic chlorophyll concentrations and epilimnetic phytoplankton production illustrate that chlorophyll is a valid indicator of eutrophication changes.

In all such studies, the rate of recovery appears related to the length of time that the lake was eutrophied, to the completeness of phosphorus reduction, to the phosphorus residence time, and probably to the sediment characteristics (Schindler et al. 1987). The P residence time is governed by water renewal and by sedimentation rates. For L226NE, the eutrophication period was moderately short (8 yr), the *P* reduction was abrupt, and the P retention time was short (< 1 yr). The recovery was therefore rapid.

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Population Dynamics of Lake Whitefish (*Coregonus clupeaformis*) During and After the Fertilization of Lake 226, the Experimental Lakes Area

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Mills, K. H., and S. M. Chalanchuk. 1987. Population dynamics of lake whitefish (*Coregonus clupeaformis*) during and after the fertilization of Lake 226, the Experimental Lakes Area. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 55–63.

Responses of an unexploited population of lake whitefish (*Coregonus clupeaformis*) to the fertilization of Lake 226 in the Experimental Lakes Area, northwestern Ontario, are described for the fifth through eighth years of fertilization (1977–80) and for three years after fertilization was terminated (1981–83). A vinyl curtain separated Lake 226 into two basins. One basin (L226NE) received additions of phosphorus, nitrogen, and carbon; the other (L226SW) received nitrogen and carbon. Lake whitefish in L226NE were faster growing, had higher condition (*k*), were more numerous, had higher survival from age 0 to age 1, had greater biomass, and had greater production than L226SW whitefish from 1977 to 1980. Some of these effects continued in 1981 and 1982 after fertilization was terminated, but only biomass differences remained by 1983.

Les auteurs traitent des effets, sur une population non exploitée de grand corégone (*Coregonus clupeaformis*), de la fertilisation du lac 226 de la Zone de lacs expérimentaux du nord-ouest de l'Ontario. L'étude a porté sur les années cinq à huit de la fertilisation (1977–1980) de même que sur les trois années suivant la fin du traitement (1981–1983). Le lac 226 était divisé en deux bassins par un écran de vinyle. Le premier bassin (L226NE) a fait l'objet d'additions de phosphore, d'azote et de carbone, le second (L226SW), d'azote et de carbone. De 1977 à 1980, les grands corégones du bassin L226NE présentaient, par rapport à ceux de l'autre bassin, une croissance plus rapide, étaient en meilleure condition (*k*) et plus nombreux et présentaient un taux de survie entre les âges 0 et 1, une biomasse et un niveau de production plus élevés. Certains de ces effets ont encore été notés en 1981 et 1982, après la fin de la fertilisation, mais seul l'écart des biomasses était encore décelable en 1983.

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The fertilization of lakes and ponds for short time periods is an established method to increase fish yield (for reviews see Vinberg and Lyaknovich 1969; Mills 1981, 1985 among others). More recently, lakes have been fertilized for longer time periods, one or more years, either to enhance growth or number of salmonids (Munro 1961; Stockner et al. 1980; Stockner 1981; Langeland 1982; Johannessen et al. 1984; Hyatt and Stockner 1985). In an earlier report, Mills (1985) described the responses of lake whitefish (*Coregonus clupeaformis*) to the first 4 yr of fertilization of Lake 226 (L226), a small two basin lake in the Experimental Lakes Area (ELA), northwestern Ontario. Lake whitefish growth, condition, biomass, survival (age 0 fish), and production were greater in the basin (L226NE) fertilized with carbon, nitrogen, and phosphorus than in the basin (L226SW) fertilized with carbon and nitrogen. Based on these results, we hypothesized that further changes would occur for lake whitefish in L226NE during 4 additional years of fertilization: (1) additional increased growth, (2) increased survival of age 0 fish to age 1 in abundant year-classes, but no difference in survival of older fish, (3) increased production, and (4) increased biomass. Evaluating the second hypothesis was particularly important. The most dramatic response of lake whitefish in L226NE to the first 4 yr of fertilization, 1973–76, was increased survival of the 1973 year-class in L226NE from fall 1973 (age 0) to fall 1974 (age 1). Because few age 0 fish were present in either basin in 1974 and 1975, it was not clear whether the survival difference was a general response for abundant year-classes or a response present only during the initial 2 yr of fertilization. Many age 0 lake whitefish were present in each basin in 1976. Increased survival of this year-class from fall 1976 to fall 1977 (age 1), would confirm the observations for the 1973 year-class.

Fertilizations of L226 were terminated in 1980. Mills (1981, 1985) suggested a 1-yr lag was present between either primary production or chlorophyll concentrations and lake whitefish production. Therefore, we hypothesized that (5) lake whitefish responses to the fertilization of L226NE would continue one or more years after fertilization was terminated.

Materials and Methods

Study Site

Lake 226 (L226) is a small, double basin lake divided into two similar basins by a vinyl sea curtain. The north basin

TABLE 1. Sampling schedule, capture methods, and catches of lake whitefish in Lake 226 from spring 1977 to fall 1983. *N* is the number of fish caught each year and x indicates sampling occurred.

	Season			Capture method		*N*	
Year	Spring (Apr.–May)	Summer (June–Aug.)	Fall (Sept.–Oct.)	Trapnets	Gillnets	NE	SW
1977	x	x	x	x	x	257	140
1978	x	x	x	x	x	367	85
1979			x		x	30	43
1980			no sampling				
1981			x[a]		x	54	
1982	x		x	x	x	282	119
1983	x		x	x	x	648	276

[a] Sampling occurred only in L226NE.

(L226NE) (8.3 ha, 14.7 m z_{max}) received additions of phosphorus ($0.34\ g \cdot m^{-2} \cdot yr^{-1}$), nitrogen ($1.81\ g \cdot m^{-2} \cdot yr^{-1}$), and carbon ($3.46\ g \cdot m^{-2} \cdot yr^{-1}$) from 1973 to 1980. The south basin (L226SW) (7.8 ha, 11.6 m z_{max}) received similar inputs of nitrogen and carbon; no phosphorus was added to this basin. Further details of the fertilization techniques are given by Cruikshank (1984) and results for other L226 biota are given by Schindler (1974), Schindler and Fee (1974), Davies (1980), Shearer et al. (1987), and Findlay and Kasian (1987).

The experimental design was disrupted during July 1979 when a forest fire burned approximately one-half of the L226 watershed. Fortunately, the fire burned equal portions of the watershed in each basin. Based on data for a similar fire in the Lake 239 watershed (Schindler et al. 1980), the L226 fire probably resulted in minimal, similar nutrient inputs to each basin during 1980, but had little impact in subsequent years. There was no detectable increase in total epilimnetic phosphorus or chlorophyll *a* concentrations in L226SW in 1980 compared to results for other years (ELA staff, unpublished data). Consequently, the forest fire probably did not confound results of the fertilization.

The experimental design was also disrupted by leakage in the vinyl curtain in 1977, 1978, and 1980. The curtain effectively segregated the L226 whitefish into two non-mixing populations from the time of its installation in May 1973 to early July 1977 when two zippers joining sections of the curtain partially separated. These openings were closed within 2 wk. This problem recurred in August 1977 but this time the opening was closed immediately. Fortunately, almost all lake whitefish in each basin were individually marked by spring 1977, and based on tag–recapture data, no whitefish moved from one basin to the other in 1977. One zipper opened again during August 1978, and was not repaired until mid-September 1978. During this period, some whitefish did move between basins, but many of these individuals could be identified because over 70% of the fish in each basin were marked. Based on captures of individually marked fish, few lake whitefish moved between basins. Furthermore, exchange between basins was almost equal (9 fish moved from L226NE to L226SW and 11 from L226SW to L226NE). Because the number of immigrants was only a small proportion of the total number in each basin (≃4% in L226SW and ≃2% in L226NE, see results section) and exchange between basins appeared equal, fish immigration probably did not confound results. Curtain leakage in 1980 was restricted to the top of the curtain where small amounts of water were transferred daily between basins. Based on tag–recapture data, no lake whitefish moved between basins in 1980.

Leakage between basins in 1977, 1978, and 1980 allowed some nutrient exchange between basins, but the effects of this leakage on the experimental design were minimal. The greatest impact in L226SW probably occurred in 1977, when leakage during mid-summer probably caused increases in primary productivity, total epilimnetic phosphorus, phytoplankton biomass, and chlorophyll *a* levels (Findlay and Kasian 1987; Shearer et al. 1987, ELA staff, unpublished data). Responses to leakage in 1978 and 1980 were not as clear as in 1977; some of the above measures increased in L226SW while others remained unchanged. Interpretation of all these changes was made even more difficult because many of these quantities increased during the same time periods in unmanipulated, reference basins (Findlay and Kasian 1987; Shearer et al. 1987; ELA staff, unpublished data). When increases in the above parameters were observed in L226SW, they were minimal relative to increases in L226NE. Therefore, leakage had little effect on the experimental design for L226.

Lake Whitefish Sampling

Lake whitefish were captured, sampled, and released using almost the same methods employed by Mills (1981, 1985) for the first 4 yr of this study. Because tag loss can bias mark-recapture abundance estimates (Arnason and Mills 1981), all lake whitefish were batch marked by systematically scarring fin rays as described in Welch and Mills (1981). Modified Carlin tage (White and Beamish 1972) were also used on fish >300 mm. The sampling schedule is summarized in Table 1. Only one overnight gillnet set was employed in each basin during 1979, and no sampling occurred in 1980. Almost all fish were returned alive to each basin except in 1979 when all fish were killed.

Data analyses

Methods used to test the five hypotheses are listed in Table 2. These are almost identical to methods used by Mills (1981, 1985) to evaluate responses during the first 4 yr of fertilization, but there are some important changes described below.

For growth analyses of lake whitefish > age 1 (hypothesis 1), Mills (1981, 1985) used back-calculated fork lengths supplemented by direct observations of tagged fish from fall 1971

TABLE 2. Hypotheses and methods used to test these hypotheses for L226NE lake whitefish. When no reference is identified, methods were identical to Mills (1981, 1985).

Hypothesis	Methods	Reference
1. Increased growth	Length at age for age 0 and 1 fish compared using t-tests	
	Analysis of covariance and adjusted means from these analyses for fish $>$ age 1	Snedecor and Cochran (1967)
	Average condition (k) compared using t-tests	
2. Increased survival of age 0 fish to age 1	Trapnet catch-per-unit-effort (C.P.U.E.)	
No change in survival of fish $>$ age 1	Jolly–Seber survival estimates from full model	Jolly (1965); Seber (1982)
3. Increased production of fish $>$ age 1	Chapman (1978) modification of Ricker method using length groups from growth analyses and abundance estimates from Jolly–Seber analyses for 1977–82 and Schnabel analyses for 1983	Seber (1982)
4. Increased biomass of fish $>$ age 1	Average of biomass present in fall of one year and amount of this present the following fall	
5. Sustained responses one year after fertilization terminated	Listed under 1–4	

to fall 1976. The back-calculations were based on measurements of the upper portion of the first pelvic fin ray. For this study we utilized the same back-calculation technique, but also used the upper portion of the first pectoral fin ray when both pelvic fins had been previously clipped. This occurred for many of the older, larger lake whitefish in 1982 and 1983. The lengths back-calculated from pectoral fins were validated in the same manner as those from pelvic fins (Mills 1981). Back-calculated lengths were compared to analogous actual lengths recorded for the individually tagged fish. No significant differences (paired t-tests, $P > 0.05$) were detected.

Assumptions of each statistical test were evaluated and satisfied prior to analysis. Lake whitefish condition (k) was not correlated either to fish age or length, so data for all fish caught in a seasonal sampling period (spring, summer, or fall) were pooled for each basin.

Because only gillnets were used to collect lake whitefish in 1979 and 1981 (Mills 1981, 1985), and selectivity of gillnets for fish with higher condition is well known (see Hubert 1983 and other standard fishery science textbooks), we compared condition of trapnetted and gillnetted fish for other sampling periods to determine whether this bias was potentially present for these dates. We found no significant differences between condition estimates based on each method for any sampling period. Therefore, we believe the 1979 and 1981 estimates are unbiased by net selectivity.

While Mills (1981, 1985) used the Jolly–Seber (Jolly 1965; Seber 1982) death-only model to estimate annual survival of lake whitefish $>$ age 1 for the first 4 yr of fertilization, we used the Jolly–Seber full model (death and emigration) to obtain these estimates for other years (hypothesis 2) because new recruits were not excluded from these later analyses. Estimates for multi-year periods, 1978–81 in L226NE and 1978–82 in L226SW, were converted to average annual estimates using Seber's (1982) delta method,

$$\hat{\phi}_i' = \hat{\phi}_i^{1/n} \text{ and}$$

$$\mathrm{SE}(\hat{\phi}_i') = \hat{\phi}^{1/n-1} \times \mathrm{SE}(\hat{\phi}_i);$$

$\hat{\phi}_i'$ is the average estimate, $\hat{\phi}_i$ is the original multi-year estimate, n is the number of years of $\hat{\phi}_i$, $\mathrm{SE}(\hat{\phi}_i')$ is the standard error of $\hat{\phi}_i'$, and $\mathrm{SE}(\hat{\phi}_i)$ is the original multi-year estimate of standard error for $\hat{\phi}_i$.

Lake whitefish production estimates (hypothesis 3) were calculated in the same manner as Mills (1981, 1985), but production was calculated for six length-groups (122–200, 201–250, 251–300, 301–350, 351–400, and 401–500 mm) instead of age-groups. We changed methods because growth was more homogeneous among members of a length class than an age class for L226 lake whitefish (Mills 1981). In addition, we used Schnabel estimates (Seber 1982) for fall 1983 because Jolly–Seber estimates were not available. To make comparisons between all years for length classes, we recalculated estimates for the 1973 to 1976 data (Mills 1981). In this study we calculated a composite abundance estimate of all length-groups for each fall sampling period and divided each in proportion to the length composition of the actual fall catch to obtain abundance estimates for individual length-groups. Gain in fish weight between fall sampling periods for 1977, 1978, 1982, and 1983 was calculated by converting back-calculated

TABLE 3. Mean lengths ($\bar{x}$) in mm and standard errors (SE) for age 0 and 1 lake whitefish. Age 0 fish are from trapnet catches. Age 1 fish are a combination of direct observations from both trapnet and gillnet catches and from back-calculations. The number of observations for each mean is N.

			Year						
			1977	1978	1979	1980	1981	1982	1983
Age 0	NE	$\bar{x}$(SE)	102(12)	—[a]	—[b]	—[b]	—[b]	77(1)	103(3)
		N	3					28	138
	SW	$\bar{x}$(SE)	89(3)	—[a]	—[b]	—[b]	—[b]	81(5)	99(4)
		N	4					16	38
Age 1	NE	$\bar{x}$(SE)	196(2)[c]	190(2)	190(3)	186(11)	203(29)	177(2)	168(3)
		N	68	14	8	3	10	65	45
	SW	$\bar{x}$(SE)	179(2)[c]	189(3)	186(4)	188(3)	176(4)	166(2)	159(70)
		N	48	23	23	16	11	72	9

[a] Sampled with trapnets and gillnets, but no age 0 fish caught.
[b] No samples available due to type of capture gear.
[c] Significant difference (t test, $P < 0.05$) between basins.

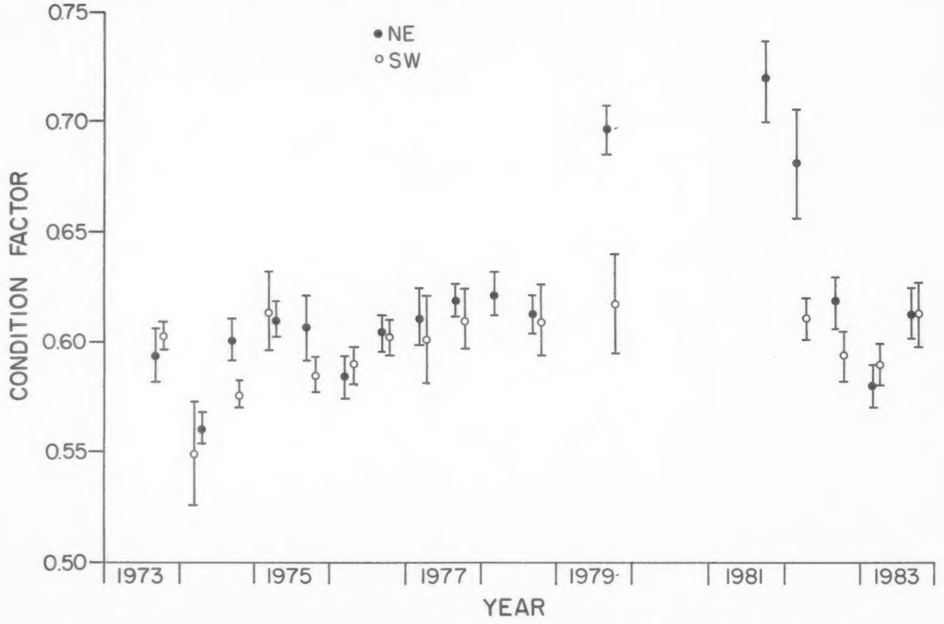

FIG. 1. Mean condition factors (k) and 95% confidence intervals for L226 lake whitefish. Data for 1973–76 are from Mills (1985).

lengths used for growth analyses to weights using the length–weight relationship from 1977 to 1983 ($\ln \mathrm{wt} = 3.18 \ln \times \mathrm{Fl} - 12.4$, $r = 0.99$, $N = 2248$). Weights for fish used to calculate production estimates for 1973 to 1976 data were calculated from the regression reported in Mills (1981, 1985).

To evaluate whether responses of lake whitefish in L226NE continued after fertilization was terminated, we used a combination of direct estimates (growth, condition, and biomass) and inferences based on year-class strengths and abundances in 1982 and 1983.

Results

Growth

For lake whitefish age 0 and 1 (Table 3), the only significant difference ($P < 0.05$) between basins occurred in 1977, when the mean length of age 1 L226NE fish was 17 mm greater than that of age 1 L226SW fish. More variability occurred between years. Age 0 lake whitefish in both basins were significantly smaller ($P < 0.05$) in 1982 than either 1977 or 1983. Age 1 fish in each basin were significantly larger ($P < 0.05$) from 1977 to 1981 than in 1982 and 1983.

For lake whitefish > age 1, L226NE fish grew faster than L226SW fish (Table 4) from 1977 to 1981 ($P < 0.01$), and 1982 ($P < 0.05$). The greatest difference between basins occurred in 1979 and 1980, and the smallest difference occurred in 1982. Growth in both basins was greater from 1977 to 1980 than in subsequent years.

Lake whitefish condition (k) was significantly greater in L226NE than L226SW in 1979 and 1982 (Fig. 1). The highest

TABLE 4. Yearly growth and fall abundance estimates of lake whitefish in L226NE and L226SW from 1971 to 1983. Growth ($\mathrm{mm \cdot yr^{-1}}$) values are adjusted means from covariance analyses using back-calculated data from this study for 1977–83 and data from Mills (1981, 1985) for 1971–76. Sample sizes are indicated in parentheses below these means. Abundance estimates are from Jolly–Seber analyses for 1977–82 and from Schnabel analyses for 1983; 95% confidence intervals are indicated by ± values in parentheses. Abundance estimates for 1973 to 1976 are from Mills (1981, 1985).

	Growth (mm)		Abundance	
	NE	SW	NE	SW
1971	13.3 (457)		900[a]	
1972	14.8 (676)		1000[a]	
1973	21.9 (445)	22.4 (343)	677	504
1974	22.0[c] (321)	14.5 (274)	914	451
1975	22.3[c] (282)	15.4 (195)	683	344
1976	16.0[c] (113)	12.9 (69)	451	223
1977	27.6[c] (329)	20.5 (136)	890 (±250)	361 (±150)
1978	24.6[c] (329)	21.0 (156)	856 (±196)	292 (±124)
1979	36.2[c] (204)	25.4 (138)	610[a]	220[a]
1980	30.6[c] (184)	22.6 (121)	440[a]	180[a]
1981	19.6[c] (189)	14.8 (135)	442 (±154)	260[a]
1982	12.6[b] (145)	10.7 (114)	451 (±88)	326 (±98)
1983	8.4 (51)	8.6 (51)	550 (±155)	336 (±110)

[a] Extrapolated estimates, based on average yearly survival from 1973 to 1982 and relative abundances of 1-yr-old fish entering the populations in these years.

[b] Significant difference between basins (analysis of covariance, $P < 0.05$).

[c] Significant difference between basins (analysis of covariance, $P < 0.001$).

condition occurred in L226NE in fall 1981. No significant differences between basins occurred in 1977, 1978, and 1983.

Survival

Based on C.P.U.E. there was greater survival of lake whitefish from age 0 (fall 1976) to age 1 (fall 1977) in L226NE than L226SW (Fig. 2). The 1977 and 1978 year-classes were sparse in each basin and there was no apparent difference in survival of age 0 fish between basins. Age 0 fish were abundant in each basin in 1982, the second year after fertilizations were terminated. There was no apparent difference between basins in survival of these age 0 fish to age 1 in 1983.

Annual survival of lake whitefish $\geqslant$ age 1 did not differ significantly between basins or years (Fig. 3).

Production and Biomass

Lake whitefish production in L226NE was more than twice that in L226SW during 1977 and 1978, but production in L226NE was only 20% higher than that in L226SW in 1983 (Table 5). The largest proportion of L226NE whitefish production occurred in the 251–300 mm length-group during 1977. This was due primarily to age 4 fish of the 1973 year-class. The large difference in production between basins in 1978 was due to the 121–200 mm and 301–350 mm length classes. These groups were composed primarily of age 2 (the 1976 year-class) and age 5 (the 1973 year-class) fish, respectively. Production differences between basins in 1977 and 1978 were due to a combination of greater abundance ($>200\%$, Table 4) and greater growth ($>30\%$ in 1977 and $>15\%$ in 1978, Table 3) in L226NE. Production in length-groups was relatively similar between basins in 1983; the greatest difference occurred for the 401–500 mm length-group. This length group was composed primarily of age 9–26 fish.

Lake whitefish production estimates for 1973–76 based on length classes (Table 5) were similar to estimates reported for each year in Mills (1981, 1985) based on age classes. Small differences between yearly estimates for each basin were due to the different methods used to calculate abundances for age-groups in Mills (1981, 1985) and for length-groups in this report.

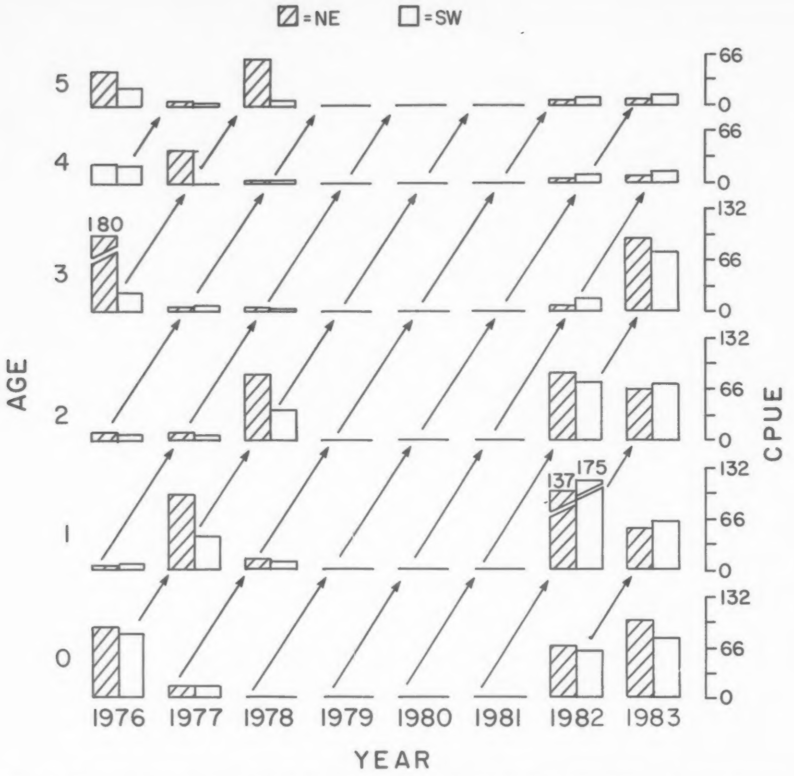

FIG. 2. Trapnet catch-per-unit-effort (C.P.U.E.) for the 1976 to 1983 lake whitefish year-classes during fall sampling periods. C.P.U.E. were adjusted to 100 trap days. Data for 1976 are from Mills (1985).

TABLE 5. Production of individual length groups, total production, and average biomass for L226NE and L226SW lake whitefish.

Length group (mm)	1973		1974		1975		1976		1977		1978		1983	
	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW
							Production for Length Groups -(kg)							
121–200	11.7	5.4	4.5	0.4	25.6	2.3	0.3	0.1	0.7	0.5	18.1	6.3	11.9	9.5
201–150	8.4	9.2	17.2	3.1	3.5	2.5	17.4	2.2	1.7	0.2	7.7	2.1	0.3	0.1
251–300	5.3	11.0	17.2	6.5	16.8	4.5	6.5	2.6	27.6	3.2	1.8	0.1	0.1	0.7
301–350	8.5	6.1	9.6	12.9	12.2	8.0	7.6	7.4	11.3	10.0	20.5	4.4	0.1	2.8
351–400	1.4	2.0	1.9	1.4	4.7	7.4	5.2	2.4	2.1	8.5	14.1	9.5	1.3	2.0
401–500	0	1.0	0.1	0.5	0.3	0.2	0.4	0.2	1.4	0.6	3.3	1.8	7.2	1.2
Total production (kg)	35.2	34.5	50.4	24.8	63.1	25.0	37.4	14.9	44.8	23.0	65.5	24.2	20.9	16.3
Average biomass (kg)	126	155	154	166	206	151	172	118	178	105	270	132	237	104

Discussion

Fertilization

Based on results for the first 4 yr of L226 fertilization, we hypothesized that L226NE lake whitefish would have (1) greater growth, (2) greater survival of age 0 whitefish to age 1 for abundant year-classes, (3) greater production, and (4) greater biomass than L226SW whitefish during the fifth through eighth years of fertilization. We also hypothesized that these responses would (5) persist for at least 1 yr after fertilization was terminated. In general, all five hypotheses were con-

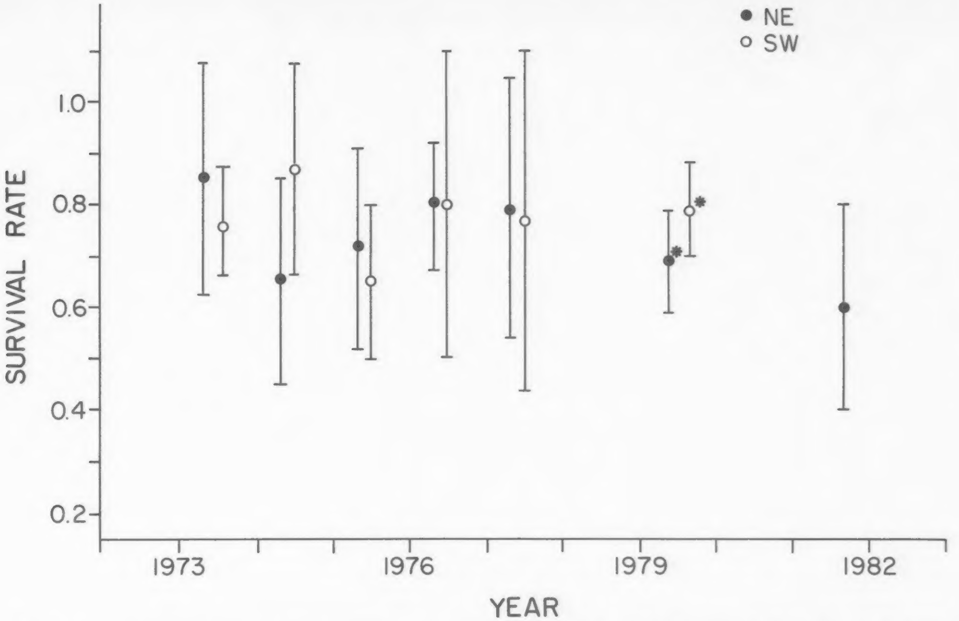

FIG. 3. Annual survival estimates ($\hat{\phi}_i$) and 95% confidence intervals for lake whitefish ≥ age 1. Estimates marked (*) are derived from multi-year estimates, as described in text. Data for 1973–76 are from Mills (1985).

firmed, although the magnitude of responses was sometimes surprising.

We had hypothesized that growth of L226NE lake whitefish would increase in L226NE during the fifth to eighth years of fertilization. This hypothesis was confirmed by results for fish > age 1 (Table 4) and by results for age 1 lake whitefish in 1977 (Table 3). Growth of L226NE lake whitefish peaked in 1979 and 1980, the seventh and eighth years of fertilization. These results are supported by the large increase in condition of L226NE lake whitefish that occurred in 1979 (Fig. 1). While no condition data are available for 1980, condition factors in each basin usually were correlated with growth of fish > age 1 (Table 4) during years in which L226 was fertilized ($r = 0.88$, $N = 14$, $P < 0.01$). Therefore, condition of L226NE whitefish in fall 1980 was probably similar to the fall 1979 value. While no significant differences occurred between lengths of age 1 whitefish between basins for years of fertilization other than 1977 (Table 3), this might be due to weak year-classes in each basin for these years (Fig. 3). An identical situation occurred during the first 4 yr of fertilization (Mills 1981, 1985). Mills (1981) predicted 3–5 yr of fertilization were necessary before a new equilibrium total phosphorus level could be achieved in L226NE, and that it would be longer before a new equilibrium level of whitefish production and biomass was reached in this basin. Therefore, we were not surprised that growth of L226NE whitefish > age 1 was highest during the seventh and eighth years of fertilization, but we were surprised that the total increase was ≃150% above 1971 (prefertilization) levels (Table 4). This is a much larger increase than reported on other studies where fertilizations were terminated after only 1–2 yr (Weatherley and Nicholls 1955; Munro 1961; Langeland and Reinertsen 1982; Johannessen et al. 1984; and others). Juday et al. (1938) fertilized a small Wisconsin lake for 5 yr and found slight increases in growth the second through fourth years of fertilization, but much greater responses of growth in perch (*Perca flavescens*) and smallmouth bass (*Micropterus dolomieui*) during the fifth year of fertilization. Unfortunately, rates of application and types of fertilizers were not uniform among years, so their results cannot be directly compared with those from L226. Both studies do show greater responses after the fourth year of lake fertilization. Therefore, it is possible that greater increases in fish growth would have occurred in other, shorter lake fertilization studies if fertilizations had continued for more than 2 yr, although other factors such as water renewal time (Dillon and Rigler 1974) could affect results.

We hypothesized that survival of age 0 lake whitefish in abundant year-classes would be greater in L226NE than L226SW and that no differences in survival of fish > age 1 would occur between basins (hypothesis 2) during fertilization. This hypothesis was accepted because of results for the 1976 year-class (Fig. 2) and results for fish > age 1 (Fig. 3). Results for the 1973 year-class were similar to those reported in Mills (1981, 1985): L226NE fish of the 1976 year-class grew faster and had higher survival than L226SW fish of the same year-class (Table 3). As in the period from 1973 to 1976 (Mills 1981, 1985), annual survival of lake whitefish > age 1 did not differ significantly between basins or years from 1977 to 1982. These estimates were similar to previous estimates for 1973–76 (Fig. 3).

Increased survival of young fish may be a general response of fish populations to lake fertilization. This occurred twice for abundant year-classes during the fertilization of L226NE. Differences between basins in survival of age 0 whitefish to age 1 did not occur after fertilizations were terminated (Fig. 2). The 1982 year-class was abundant in each L226 basin, but no survival difference was apparent between basins in 1983 (age 1). Baronov et al. (1973) reported similar results for

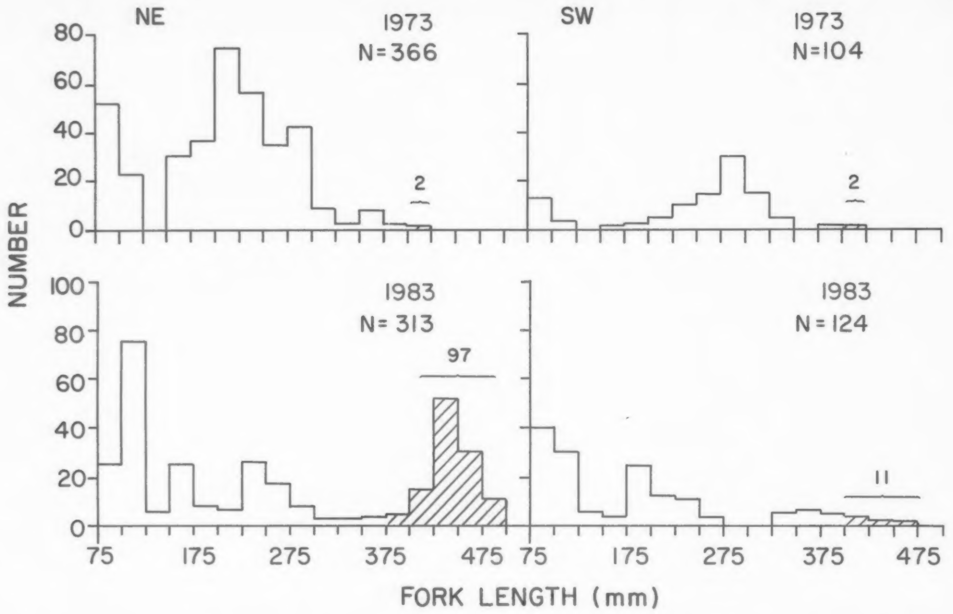

FIG. 4. Length frequency distributions of the lake whitefish catch in fall 1973 and fall 1983. The number of fish ≥400 mm is indicated above the cross-hatching.

coregonid fishes stocked in fertilized lakes in the Soviet Union. This response is also consistent with results of Langeland (1982), who found abundant year-classes of Arctic char (*Salvelinus alpinus*), and Johannessen et al. (1984), who found increased catches of small brown trout (*Salmo trutta*), during the fertilization of other small lakes.

Lake whitefish production in L226NE was greater than that in L226SW in 1977 and 1978 (Table 5) and similar results probably occurred in 1979 and 1980. Fish production estimates are based on fish growth and abundances (Chapman 1978). Growth of lake whitefish > age 1 was greater in L226NE than L226SW in both 1979 and 1980 (Table 4). Using mark–recapture abundance estimates for 1978 and 1982 in each basin and assuming no apparent differences in recruitment between basins from 1979 to 1981 (Fig. 3), we extrapolated abundance estimates for fall in 1979, 1980, and 1981 (Table 4). More lake whitefish were probably present in L226NE than L226SW for these years. Therefore, production was probably much greater in L226NE than L226SW in 1979 and 1980, and hypothesis three is confirmed.

Average lake whitefish biomass was higher in L226NE than L226SW in 1977 and 1978, and this probably continued in 1979 and 1980, confirming hypothesis four. Based on growth, extrapolated abundances, and biomass present in each basin in 1978 and 1983 (Tables 4 and 5), lake whitefish biomass in L226NE was probably greater than 200 kg in L226NE while biomass in L226SW was probably similar to that in other years (≃ 133 kg).

The increase in growth of L226SW lake whitefish > age 1 (Table 4) between 1973–76 and 1977–80 is difficult to explain. Growth of L226SW fish during the latter years was greater than that of L226NE lake whitefish during the initial years of fertilization. The increased growth may be related to phosphorus leakage from L226NE to L226SW caused by zipper failures described earlier, or more diffuse, gradual leakage in the sediments under the curtain. The lag from chlorophyll *a* or primary production to whitefish production (Mills 1981, 1985) could prolong the effect of such leakages. The increase might also be related to the low lake whitefish density in L226SW. In general, there were fewer lake whitefish present in L226SW during 1977–80 than 1973–76, but differences in abundance cannot entirely account for the increased growth. For example, even though growth of L226SW fish was similar in 1973 and 1980, more than twice as many fish were present in 1973. Other factors, such as annual variability in growth, may also be important.

Postfertilization

We hypothesized that effects of fertilization in L226NE would continue one or more years after nutrient additions were terminated. This hypothesis (hypothesis five) was confirmed, but one effect continued to the third year after additions ceased. Growth of lake whitefish > age 1 was significantly greater in L226NE than L226SW in both 1981 and 1982 (Table 4), but the difference between basins gradually declined from 1981 (8 mm) to 1982 (2 mm). Condition estimates for 1982 and 1983 in L226NE (Fig. 2) parallel the progressive growth decline in this basin. Based on growth and abundance estimates (Table 4), lake whitefish production was probably much greater in L226NE than L226SW in 1981 and 1982. There was little difference in lake whitefish growth, condition, and production between basins in the third year after additions had ceased. Unlike other parameters, effects on biomass were present to the third year of fertilization (Table 5). Average biomass was higher in 1983 than 1973 to 1977 in L226NE.

This is not surprising. Annual survival of lake whitefish > age 1 was relatively constant during all years of study in each basin. Greater growth in L226NE than in L226SW over a 7-yr period resulted in many large fish (>400 mm in length and >1 kg in weight) in L226NE, and these large fish were still present in 1983, 3 yr after fertilization was terminated (Fig. 4). The elevated biomass in L226NE will probably persist in future years because of the high survival of L226 lake whitefish.

Conclusion

We believe the most striking response of lake whitefish to fertilization of L226NE was increased survival of age 0 fish. The influence of this change was reflected in greater abundance in L226NE than L226SW and was a major factor for production differences between basins. Mills (1981) suggested it might take 6 successive years of fertilization before a new equilibrium biomass of lake whitefish occurred in L226NE. We now believe that a new equilibrium had not been reached after 8 yr of fertilization. Growth of L226NE fish in the seventh and eighth years of fertilization was higher than previous years. Lake whitefish production and biomass probably also increased during these years. These parameters may have increased further if fertilization had continued beyond 1980. Similarly, even though growth, condition, and production had declined in L226NE after fertilization was terminated, the lake whitefish population was still very different from what it was when fertilization commenced in 1973. It is difficult to speculate about the number of years this effect will persist. Many large lake whitefish are still present in L226NE in 1985 (K. H. Mills, unpublished data).

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Detection of Handling Mortality and its Effects on Jolly–Seber Estimates for Mark–Recapture Experiments

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Handling mortality occurs in mark–recapture experiments if animals handled and released in a given sample have a higher mortality rate than animals that were alive but not sampled. This violates the assumption of equal survival required for forming the Jolly–Seber estimates of population abundance, survival, and recruitment. We show that handling mortality can produce very large biases in these estimates, and we develop a test to detect it. We investigate the power of this test and find that quite large biases can be produced at handling mortality rates that are too low to be detected. We recommend methods to prevent handling mortality from occurring in fish sampling experiments and methods to reduce bias in the estimates. The test and the bias corrections are applied to mark–recapture data for a lake whitefish (*Coregonus clupeaformis*) population and to data from simulated mark–recapture experiments. Because of unavoidable inadequacies in the detection and reduction of bias due to handling effects, we strongly recommend that fisheries biologists take great care to prevent its occurrence.

Il y a mortalité par manutention au cours des essais par marquage et recapture lorsque les animaux manipulés et remis en liberté d'un échantillon donné présentent une mortalité plus élevée que les animaux non échantillonnés. Cette mortalité vient à l'encontre de l'hypothèse des taux de survie égaux à la base des estimations de Jolly–Seber de l'abondance, de la survie et du recrutement des populations. Les auteurs montrent que la mortalité par manutention peut fausser de façon très importante ces estimations et élaborent un test pour la déceler. Il est apparu, après analyse du test, que de très importants écarts peuvent être produits par des taux de mortalité de manutention trop faibles pour être décelés. Les auteurs recommandent des méthodes visant à prévenir cette mortalité au cours des échantillonnages de poisson ainsi que des méthodes visant à réduire le biais des estimations. Le test et les corrections de biais sont appliqués à des données de marquage–recapture obtenues d'une population de corégones de lacs (*Coregonus clupeaformis*) ou par essais simulés. Étant donné les erreurs inévitables de détection et de réduction du biais résultant de la manutention, les auteurs recommandent fortement aux biologistes des pêches de veiller le plus possible à les éviter.

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Since its introduction in 1965, the Jolly–Seber (Jolly 1965; Seber 1965) method has gradually replaced earlier models for estimating abundance, survival, and number of new recruits in multiple mark–recapture experiments. The technique, when applied to a fish population, involves taking samples of fish from a well-defined population at discrete, though not necessarily equally spaced, sample times. The notation used here for the results of the experiment is explained in detail in Arnason and Mills (1981) but can be summarized as follows: of the n_i fish taken in the i-th sample, the u_i unmarked fish are given a mark, usually a unique identifier, and the capture of each of the m_i previously marked fish is recorded. Any of the n_i fish that die or are removed are called "losses-on-capture"; they result in the return of s_i ($\leq n_i$) marked fish to the population. After K samples, knowledge of the capture history of each marked fish permits the computation of r_i, the number of fish recaptured at least once after time i out of the s_i releases at time i. The statistics n_i, m_i, s_i, and r_i ($i = 1 \cdots K$) are sufficient for forming estimates of the population parameters of interest: N_i, the population size at time i; ϕ_i, the proportion surviving from time i to time $i+1$; and B_i, the number of immigrants or new recruits entering the catchable population between i and $i+1$. One can also estimate M_i, the number of marked animals alive at the time the i-th sample is taken and π_i, the probability that an animal is taken in the i-th sample. The estimates and their standard errors (designated, for example, by $\hat{N}_i$ and $S(\hat{N}_i)$) were developed by Jolly (1965) and Seber (1965). The former gave estimates for two models of wide biological applicability: the full model allows N_i, B_i, and ϕ_i to vary with each sample time or period, while the death-only model assumes the B_i are all equal to zero but allows N_i and ϕ_i to vary with i.

Both models require rather rigorous assumptions. The two most important are requirements of equal catchability and equal survival. The first means that every fish alive in the population when the i-th sample is taken has the same probability of being captured. The second means that every fish alive just after the i-th sample is returned has the same probability of survival to the next sample time. The failure of these assumptions is termed heterogeneous capture or survival. Heterogeneity may be due to differences among fish in observable factors (e.g. age, sex, species), unobservable factors (e.g. behavioral differences), or capture history (e.g. marked or unmarked). Assumption failures generally cause bias in the estimates and in their standard errors, but the degree of bias is a function of when, why, and by how much the assumptions failed, and different estimates from the same or different models are affected to varying degrees. These effects are reviewed by Seber (1982, Chapter 5) and Arnason and Baniuk (1978, Chapter 5). Cormack (1972) showed how to assess these effects heuristically. One conclusion of his analysis is that the Jolly−Seber abundance estimate ($\hat{N}_i$) is robust to survival heterogeneity provided survival does not depend on capture history. This is also true of the survival estimate ($\hat{\phi}_i$), although it estimates a "gross" survival rate over all, or some subset, of the population, because survival heterogeneity means that different survival rates occur in two or more subsets of the population. Such "gross" estimates may be of little biological use even if they are unbiased. In such situations, the biologist is usually interested in the survival rate of specific subgroups: e.g. age-specific survival or "natural" survival of animals unaffected by mark status or other sampling artifacts.

Survival heterogeneity was noticed in a study of responses of lake whitefish (*Coregonus clupeaformis*) to whole-lake fertilization (Mills 1981, 1985). Early in the study, it became obvious that some fish died, shortly after release, due to the handling involved in capturing and/or marking them. This greatly increased the Jolly−Seber estimates of population size at two sample times to implausibly large values. Fortunately, the circumstances made it clear that these estimates were biased and allowed the cause of the bias to be identified. No immigration or emigration of fish occurred and new recruits, identified by their age, were excluded from the analyses. Because only deaths could occur, the abundance estimates should have shown a downward trend throughout the study. Abundance estimates did not follow this trend and some survival estimates over 100% were also produced. The reason for these obvious biases was evident from the recapture rates, r_i/s_i, at the two times. If a population with high, roughly constant, monthly survival is sampled frequently with constant effort, these rates should be similar from one sample to the next. These circumstances applied to our study, yet the r_i/s_i at the two times were only half the values at most other times. This could only be accounted for by an additional source of mortality, due to the capture and/or tagging of the fish at these two times, that caused the released fish to die at a higher rate than fish not caught at these two times. The recapture rates were so low that this source of mortality could not have been due to tagging alone; it must have affected both the u_i animals that were captured and newly tagged and the m_i animals that were only captured at these times. This source of additional mortality is called handling mortality (Brownie and Robson 1983).

It is important to differentiate between handling mortality and type-1 loss, a similar form of handling-induced survival heterogeneity. Handling mortality results at time i when all fish captured and released at that time have, due to the stress of capture and handling, a lower survival rate to time $i+1$ than fish not captured at time i. By contrast, type-1 loss is due to the process of marking and so affects only the unmarked fish which are then marked and released. Both effects can occur simultaneously, resulting in an even lower survival rate for newly marked fish. If either effect persists only to time $i+1$, it is called a one-period effect; that is, handled or newly marked fish that *do* survive to just before time $i+1$ have, from then on, the same capture and survival probabilities as fish not handled at time i. Because both effects result from handling, it would be better to name handling mortality "capture mortality" and type-1 loss "marking mortality" but both names are well established in the literature, so we retain the former designations.

Investigations of both effects can be found in the statistical literature. Robson (1969) developed a model for one- and two-period marking effects, which he called type-1 and type-2 loss, and tests to detect the presence of these effects. He derived an unbiased abundance estimator for a full model allowing for type-1 loss. This estimate is the same as one developed by Manly (1969) to allow for age-dependent survival. Pollock (1975) developed a general system of models, tests, and estimates allowing for multi-period effects of marking on both catchability and survival in full or death-only models. Recently, Brownie and Robson (1983) have developed mark−recapture methods that allow for band-resighting; that is the identification of individual marks on previously banded animals without their capture and handling. They developed a number of models for such data. Their model R2 allows for type-1 loss and handling effects. The Brownie and Robson study is the only consideration of handling effects in the statistical literature. Their estimators of handling effects cannot be applied to ordinary mark−recapture experiments because they require resightings of non-handled animals.

Type-1 loss is also well documented in the fisheries literature (Ricker 1975; Wydaski and Emery 1983), but we are not aware of any comprehensive discussion of handling mortality. The standard practice, recommended in many fishery methods manuals (Ricker 1975; Bagenal and Tesch 1978), is to hold newly tagged fish in enclosures for short periods of time to assess type-1 loss.

In other mark−recapture studies, the circumstances that made handling-mortality biases so obvious in our study may not exist. As a result, biased estimates might be erroneously attributed to a sudden upsurge in recruitment or immigration, or to a sudden increase in the natural survival rate. Because methods of sampling fish are more stressful than methods for most other animals, fish are more likely to experience handling mortality. Therefore, it is especially important for fisheries workers to understand the magnitude and pattern of bias induced by handling mortality in Jolly−Seber estimates of abundance, survival, and recruitment. In this paper, we give analytic formulae for these biases, given a simple but biologically reasonable model for the way handling mortality occurs. We develop a test for detecting handling mortality and examine the properties of the test. We suggest ways of reducing the bias, both by improvements to sampling methodology and by modifications to the Jolly−Seber analyses of data. Finally, we illustrate these procedures by presenting a thorough analysis of the data that motivated this study.

All of the analyses carried out in this paper were done using the POPAN computer system of Arnason and Baniuk (1978). POPAN has several subsystems, called paragraphs, which we indicate throughout the paper in capital letters. Sampling data with known mechanisms and parameters, including handling

TABLE 1. Asymptotic relative bias (ARB) of Jolly–Seber estimates from the full (F) and death-only (D) models when handling mortality at rate $(1 - \theta_h)$ occurs at time h only, when capture rate is π_h. ARB is zero for estimates at all sampling times other than those shown.

Result	Estimate	Estimate definition	Models	ARB
1	$\hat{N}_h$	Abundance at time h	F,D	$(1 - \pi_h)(1 - \theta_h)/\theta_h$
2	$\hat{\phi}_{h-1}$	Survival rate from $h - 1$ to h	F,D	
3	$\hat{M}_h$	Marked animals alive at h	F	
4	$\hat{B}_{h-1}$	Recruits entering in $h - 1$ to h	F	
5	$\hat{\phi}_h$	Survival rate from h to $h + 1$	F,D	$-(1 - \theta_h)$

mortality, were generated and analysed by SIMULATE. ANALYSIS computes full and death-only model estimates without or with allowance for type-1 loss. We refer to the latter as the "modified" estimates, following the designation of Arnason and Baniuk (1978, Section 5.2.4), where their derivation from the work of Robson (1969) and others is discussed. The SELECT paragraph permits selection of subsets of the animals and of the sample times, including the ability to OMIT one or more sample times. The STATISTICS paragraph includes a very general but self-explanatory set of phrases for obtaining counts of fish in the various sample history classes needed to construct tests or form estimates. We will also use these phrases in this paper to define counts and history classes; for example, the statistic s_i is defined by the class SEEN AT (I) AND NOT LOST AT (I), where the POPAN verb 'SEEN' means 'captured'. Finally, some of the analyses of the study data were done using a new release of POPAN (Arnason and Schwarz 1987) that includes the analyses of Jolly (1982) which allow for constant monthly survival.

Effects of Handling Mortality

To assess the bias introduced by handling mortality, we adopt the following simplest model for its occurrence. Suppose that, in a standard Jolly–Seber experiment with no losses on capture, there is just one sample time, h, when handling mortality occurs. At all other sampling times, the equal survival assumption holds. We assume that equal catchability holds at all times and that handling mortality is a one-period effect. The $N_h - n_h$ animals that are not handled at time h have survival rate ϕ_h, while the s_h animals that were handled and returned have an additional independent risk of mortality of $1 - \theta_h$. Thus, the probability that one of the s_h handled animals survives to time $h+1$ is $\phi_h\theta_h$. Clearly, this simplest model can be extended to allow a different handling mortality, $1 - \theta_i$, at each sample time. We shall refer to this as the general handling mortality model. Given the simple model, it is possible to work out the asymptotic relative bias (ARB) using the methods of Carothers (1973) or Arnason and Mills (1981). The ARB is the difference between the expected value of an estimate and its true quantity, relative to that true quantity. The notation for this, using the abundance estimates as an example, is $\mathrm{ARB}(\hat{N}_i) = (E(\hat{N}_i) - N_i)/N_i$. The development of the ARB formulae are outlined in Appendix A.

Table 1 summarizes the ARB results. Bias occurs only in those estimates whose definition involves the single sample time, h, at which handling mortality occurred. There is no bias in estimates at any other time. The ARB of all estimates at h, or over $h - 1$ to h, share a common formula (results 1–4 in Table 1). Rather surprisingly, the common ARB is proportional to the fraction *not* handled at time h, $1 - \pi_h$. This must be so because, if all animals were handled at time h (i.e. $\pi_h = 1$), then we would again have homogeneous survival, at rate $\phi_h\theta_h$, and hence unbiased estimates. The ARB is also proportional to the odds ratio $(1 - \theta)/\theta$ for handling mortality. This can result in huge biases in all these estimates, even at quite moderate handling mortalities. Some numerical examples are given in Table 2.

The ARB results for the two survival rates (results 2 and 5 in Table 1) give a characteristic pattern that may be a useful diagnostic for the presence of handling mortality at a single time. $\hat{\phi}_{h-1}$ is a gross overestimate, and will usually be inadmissible (i.e. >1.0), followed by an unusually low (negatively biased) $\hat{\phi}_h$. A consequence of result 5 is that $E(\hat{\phi}_h) = \theta_h\phi_h$; that is, $\hat{\phi}_h$ is an unbiased estimator of the survival rate of the handled animals. Moreover, $S(\hat{\phi}_h)$ appears from simulation investigations to be an unbiased estimate of $S(\widehat{\phi_h\theta_h})$, the true standard error of the estimate. While $\hat{\phi}_h$ gives an interpretable estimate, it is not a very useful one. It does not permit estimation of the "natural" survival rate, ϕ_h, nor of the gross survival rate, $\phi_h[1 - \pi_h(1 - \theta_h)]$, but rather underestimates both of these quantities.

A number of simulations of the handling effects model were carried out using SIMULATE. These confirmed the ARB results for a wide range of parameter values and showed that none of the standard error formulae, except $S(\hat{\phi}_h)$, gave useful results for any of the estimates in Table 1. They always gave overestimates of the true standard error of the estimate, but gave underestimates of the root mean squared error.

These results extend easily to the general model where a different handling mortality occurs at each sample time h. In this case, results 1 and 3 in Table 1, for the bias of $\hat{N}_h$ and $\hat{M}_h$, still hold. Results 2 and 5, for the bias of $\hat{\phi}_{h-1}$ and $\hat{\phi}_h$, hold only if there is no handling mortality at $h - 1$ and $h + 1$. If there is handling mortality for a series of sample times, say $h = i, i + 1, \ldots j$, then result 2 applies to $\hat{\phi}_h$ at $h = i - 1$ and result 5 applies at $h = j$ but the bias will usually be less than that given by result 2 for $h = i, \ldots j - 1$. In fact, as we show in Appendix A, $\hat{\phi}_h$ is an unbiased estimate of gross survival in the (biologically unlikely) event that π_h and θ_h are constant over this range.

These biases indicate that none of the usual Jolly–Seber estimates are reliable if handling mortality is present at all times. However, one might hope to derive new, unbiased estimation formulae for the general handling effects model, as Robson (1969) and Pollock (1975) did for marking effects

TABLE 2. Expected value of time 3 abundance estimates, $E(\hat{N}_3)$; time 2 to 3 and time 3 to 4 survival estimates, $E(\hat{\phi}_2)$, and $E(\hat{\phi}_3)$, when handling mortality at rates shown occurs at time 3. The results apply to a 6-sample experiment on an initial population of 600, subject to death only at rate 25% and capture at rate 22% at each sample time ($N_1 = 600$, $\phi_i = 0.75$, $\pi_i = 0.22$, $i = 1 \ldots 6$). Relative bias shown applies to $\hat{N}_3$ and $\hat{\phi}_2$. Relative bias of $\hat{\phi}_3$ can be judged by comparison of $E(\hat{\phi}_3)$ to the true (gross) rate shown below $E(\hat{\phi}_3)$. True values of N_3 and ϕ_2 are shown under handling mortality = 0. Test results over 20 independent simulations at each level of handling mortality show mean ($\bar{z}$) and SD (s_z) of the test statistic. β_{50}, β_{10}, and β_5 show the percent significant (out of 20) at the 50, 10, and 5% levels. β_{50} is the percentage of positive z values.

	Percent handling mortality: $100(1-\theta)$								
	0	20	33	47	60	67	73	87	93
Survival rate of handled animals:	0.75	0.60	0.50	0.40	0.30	0.25	0.20	0.10	0.05
% relative bias: $100(1-\pi)(1-\theta)/\theta$	0	20	39	68	117	156	215	507	1092
$E(\hat{N}_3)$	394	471	547	662	884	1008	1238	2390	4693
$E(\hat{\phi}_2)$	0.75	0.90	1.04	1.26	1.63	1.92	2.36	4.55	8.94
$E(\hat{\phi}_3)$	0.75	0.60	0.50	0.40	0.30	0.25	0.20	0.10	0.05
(true)	(0.75)	(0.72)	(0.70)	(0.67)	(0.65)	(0.64)	(0.63)	(0.61)	(0.60)
Test results:									
$\bar{z}$	−1.11	−0.26	0.21	0.51	1.40	2.03	2.04	3.01	3.61
s_z	1.03	0.87	1.14	0.72	0.95	0.88	0.80	0.63	0.89
β_{50}	15	35	55	75	90	100	100	100	100
β_{10}	0	5	15	10	60	85	85	100	95
β_5	0	5	15	5	45	70	60	100	95

models. Unfortunately, a general result of Robson (1969) proves that this cannot be done. He showed that heterogeneity of survival due to differences in capture history can occur, and still permit estimation of population abundances and survival, provided that capture histories can be grouped into classes such that survival is homogeneous within each class. There are some constraints on these classes, however, if all class sizes, and hence class-specific survival and total abundance, are to be capable of estimation. The key condition for identifiability is (Robson 1969):

> there is at least one class that includes a history involving capture at time i and another history involving no capture at time i, for every $i \geq 2$.

In Table 3(a), we define these history classes for the type-1 loss model, where identifiability is possible and, by contrast, those for the general handling effects model where the identifiability condition fails. Note that in both models, every possible capture history to time i is accounted for. For type-1 loss, it is the third class (of fish whose survival rate is ϕ_i) that satisfies the condition. For the handling effects model, class 1 and 3 fail the condition because all histories involve no capture at time i; class 2 (of fish whose survival rate is $\phi_i\theta_i$) fails because all histories involve capture at i. A model with both type-1 loss and handling mortality would have 4 classes, the first history in class 2 forming a separate class (subject to type-1 loss and handling effect), and would also be non-identifiable.

A Test for Handling Mortality

Failure of the identifiability condition also means that no unbiased, efficient test can be constructed for detecting handling mortality. We have, however, developed an ad hoc test that seems to be the best test achievable. Bias in a test results from a discrepancy between the nominal and actual significance levels, and efficiency relates to the power of the test. These terms are discussed in statistics texts (e.g. Conover 1971, Section 2.3–2.4) but for our purposes they can be explained as follows. An unbiased test for handling mortality, carried out at the α% significance level, will reject the hypothesis of 'no handling mortality' α% of the time when there is, in fact, no handling mortality. Our test is conservatively biased in that it will reject this hypothesis less than α% of the time. A test has power β% if it rejects the hypothesis of 'no handling mortality' β% of the time when handling mortality is, in fact, occurring. β is an increasing function of α, the nominal significance level used, and of $(1 - \theta_i)$, the magnitude of handling mortality, but it also depends on the other parameters of the model (N_i, π_i, ϕ_i, etc.). We cannot be sure that our test is efficient in the sense that no other test is more powerful for the same α and parameter values. The test, and its bias and efficiency properties, are now developed assuming the simple model for handling mortality.

The test for the presence of handling mortality at a single sample time, h, can be carried out by comparing two proportions: $P_1 = R.1/S.1$, the proportion of animals recaptured after time h out of those seen at $h - 1$ but not handled at time h; and $P_2 = R.2/S.2$, the proportion of recaptures after time h out of those handled at time h. The statistics $R.1$, $S.1$, $R.2$, and $S.2$ are defined more formally, with example histories, in Table 3(b) (handling mortality model). Computationally, the test is the one-tailed contingency table test described by Conover (1971, section 4.1) for testing the null hypothesis that $P_1 \leq P_2$ versus the alternative $P_1 > P_2$. The proportions here refer to the expected proportions rather than the observed proportions. The test statistic z is computed as:

$$(1) \qquad z = d/\mathrm{SE}(d)$$

where

$$d = (R.1/S.1) - (R.2/S.2)$$

and

$$\mathrm{SE}(d) = \left(\left(\frac{1}{S.1} + \frac{1}{S.2}\right)\left(\frac{R.1 + R.2}{S.1 + S.2}\right)\left(1 - \frac{R.1 + R.2}{S.1 + S.2}\right)\right)^{1/2}$$

TABLE 3(a). Capture history classes for 2 models of history-dependent survival. Survival from sampling time I to I + 1 is assumed homogeneous among fish whose histories belong to the same class but may differ between fish in different classes. Complete history types for a $K = 6$ sample experiment with I = 3 are shown as a sequence of 6 events where 0 means 'not seen' (captured), 1 means 'seen', and — means either 0 or 1. Capital letters are used for POPAN STATISTICS phrases.

	TYPE-1 LOSS MODEL							HANDLING MORTALITY MODEL						
		History type							History Type					
Class	Class Definition	1	2	3	4	5	6	Class Definition	1	2	3	4	5	6
1	FIRSTSEEN AFTER (I)	0	0	0	—	—	—	FIRSTSEEN AFTER (I)	0	0	0	—	—	—
2	FIRSTSEEN AT (I) (subject to type-1 loss effect at I = 3)	0	0	1	—	—	—	SEEN AT (I) (subject to handling effect at I = 3)	0	0	1	—	—	—
									0	1	1	—	—	—
									1	0	1	—	—	—
									1	1	1	—	—	—
3	FIRSTSEEN BEFORE (I)	0	1	0	—	—	—	SEEN BEFORE (I) AND NOT SEEN AT (I)	0	1	0	—	—	—
		0	1	1	—	—	—		1	0	0	—	—	—
		1	0	0	—	—	—		1	1	0	—	—	—
		1	0	1	—	—	—							
		1	1	0	—	—	—							
		1	1	1	—	—	—							

TABLE 3(b). Counts needed to test for marking (type-1 loss model) or capture (handling mortality model) effects on survival from time I to I + 1. Each count is the number of fish whose capture history satisfies the given class definition, specified as in Table 3(a) by its POPAN STATISTICS phrase. If neither effect occurs, one expects $R.1/S.1 = R.2/S.2$ (type-1 loss model) or $R.1/S.1 \leqslant R.2/S.2$ (handling mortality model). If the effect occurs, in its respective model, the group 2 recapture rate is lowered. History types for each class are given for $K = 6$ and I = 3 using the same notation as in Table 3(a) but with (**) to indicate times during which at least one capture occurs.

	TYPE-1 LOSS MODEL							HANDLING MORTALITY MODEL						
		History type							History type					
Count Group	Class Definition	1	2	3	4	5	6	Class Definition	1	2	3	4	5	6
$R.1$	SEEN BEFORE (I) AND SEEN AT (I) AND SEEN AFTER (I)	(*	*)	1	(*	*	*)	SEEN AT (I-1) AND NOT SEEN AT (I) AND SEEN AFTER (I)	—	1	0	(*	*	*)
$S.1$	SEEN BEFORE (I) AND SEEN AT (I) AND NOT LOST AT (I)	(*	*)	1	—	—	—	SEEN AT (I-1) AND NOT SEEN AT (I) AND NOT LOST AT (I-1)	—	1	0	—	—	—
$R.2$	FIRSTSEEN AT (I) AND SEEN AFTER (I)	0	0	1	(*	*	*)	SEEN AT (I) AND SEEN AFTER (I)	—	—	1	(*	*	*)
$S.2$	FIRSTSEEN AT (I) AND NOT LOST AT (I)	0	0	1	—	—	—	SEEN AT (I) AND NOT LOST AT (I)	—	—	1	—	—	—

To judge significance, z is treated as a standard normal variate; e.g. it is significant at the (nominal) 5% level if $z > 1.64$, and at the 10% level if $z > 1.28$.

If no handling mortality occurs, then Conover's null hypothesis will be true ($P_1 \leqslant P_2$) because the $S.1$ animals released at $h-1$ may die during h-1 to h, but if they live, they have the same chance of recapture as the $S.2$ group 2 animals. However, if handling mortality occurs during h to $h + 1$ at a sufficiently high rate, recapture of the group 2 individuals will be low enough that the alternative hypothesis is true. However, the null hypothesis $P_1 \leqslant P_2$ may be true even when handling mortality is present. This leads to low power until handling mortality is large enough that $P_1 = P_2$. At this point z actually has a standard normal distribution, if $S.1$ and $S.2$ are reasonably large, and the power of the test equals the nominal significance level. The magnitude of handling mortality that produces $P_1 = P_2$ is derived in Appendix B and is given in Table 4 for a range of ϕ_{h-1} and π_h values. The main conclusion of these results is that, unless survival rates per sample interval are very high (>90%), handling mortality must be quite high (>20%) if the test is to have any appreciable chance of detecting the presence of handling mortality. At all values, handling mortality is producing large, often huge, biases in the estimates before it is detectable by the test. Some examples of the actual power of the test, at several handling mortalities, were

TABLE 4. Values of % handling mortality, $100(1 - \theta_i)$, above which the test has at least an α% chance of giving a significant result at the α% level, for various values of ϕ_{i-1} and π_i (i.e. $\beta \geqslant \alpha$; see Appendix B for derivation). The relative bias of $\hat{N}_i$ and $\hat{\phi}_{i-1}$, as given by the formula $(1 - \pi_i)(1 - \theta_i)/\theta_i$, is shown on the right of the table, because the ARB is determined by θ_{i-1} alone when $\alpha = \beta$.

Survival rate over $(i - 1, i)$: ϕ_{i-1}	Capture rate at time i: π_i					Relative bias
	0.1	0.2	0.3	0.4	0.5	
	% handling mortality required for $\beta \geqslant \alpha$					
0.5	53	56	59	62	67	1.00
0.6	43	45	49	53	57	0.67
0.7	32	35	38	42	46	0.43
0.8	22	24	26	29	33	0.25
0.9	11	12	14	16	18	0.11

TABLE 5. Analysis of a single simulated data set from a death-only model with handling mortality of 67% at time $h = 3$ and other parameters as for Table 2. True values give actual abundance (N_i) and survival (ϕ_i) at each sample time i, accounting for any removals of animals or deletion of sample times due to the analysis method. SE is the standard error of the estimate.

Method	True Values			Death-only model estimates				Modified death-only estimates			
	i	N_i	ϕ_i	$\hat{N}_i$	(SE)	$\hat{\phi}_i$	(SE)	$\hat{N}_i$	(SE)	$\hat{\phi}_i$	(SE)
1. Data as is (359 histories)	1	700	0.77	667	(58)	0.77	(0.11)	—	—	—	—
	2	539	0.74	511	(54)	1.86	(0.30)	452	(93)	2.50	(0.57)
	3	401	0.68	953	(271)	0.25	(0.08)	1131	(588)	0.19	(0.11)
	4	272	0.71	235	(38)	0.85	(0.28)	218	(46)	1.08	(0.47)
	5	192	0.80	199	(56)	—	—	236	(96)	—	—
2. Deleting all (88) histories with capture at time 3	1	612	0.74	594	(70)	0.68	(0.13)				
	2	451	0.55	401	(60)	0.54	(0.13)				
	4	246	0.71	218	(38)	0.75	(0.25)				
	5	174	0.79	164	(45)	—	—				
3. Omitting sample time 3 (43 histories deleted, 88 captures ignored)	1	700	0.77	738	(86)	0.65	(0.12)	—	—	—	—
	2	539	0.50	484	(71)	0.49	(0.11)	371	(90)	0.64	(0.24)
	4	272	0.71	235	(38)	0.85	(0.28)	236	(60)	0.97	(0.47)
	5	192	0.80	199	(56)	—	—	229	(93)	—	—

produced by simulation and are reported in Table 2. Power was poor (<40%) for handling mortalities less than 50% (relative bias <100%) even though there was large bias in all the $\hat{N}_3$ and $\hat{\phi}_2$ at these values. As the test results in Table 2 illustrate, the power rises rapidly once handling mortality increases beyond the point required for $P_1 = P_2$ (Table 4). This occurs at a handling mortality of 30% for the populations used in Table 2.

This test was designed to detect a handling effect at a single sample time. It will have poor power at $i = h + 1$ if there are similar capture and handling mortality rates at h and $h + 1$, as can be deduced from the general model ARB of the $\hat{\phi}_h$ given in the previous section. Thus, if a handling effect is detected or suspected at time h, the test for handling mortality at the next time, $h + 1$, should be conducted ignoring captures at time h. In practice, one simply omits captures at time h from all histories and repeats the test.

Discussion of the Test and Bias Reduction Measures

The test proposed above is not very effective at detecting handling mortality at low or even moderate rates, but in our experience with fisheries data, handling mortality rates are either negligible or extremely high. Usually, an environmental factor such as high water temperature, rough handling, or excessive use of anaesthetic is the cause of handling mortality. With other organisms, a lower handling mortality may fall in the dangerous zone where it inflicts serious bias in estimates but is not objectively detectable. Nevertheless, when survival rates are high, as they often are in fish experiments, the power of this test will not be much worse than that of the more efficient unbiased tests for marking effects (Robson 1969; Pollock 1975). We recommend that the test be carried out at a nominal 10% or even 20% significance level rather than the more usual 5% level because the actual significance will be appreciably lower than the nominal level and because it lessens the chance of type II errors (failing to detect handling mortality when it is present). We also recommend carrying out Robson's (1969) test for type-1 loss, because type-1 loss and handling mortality are likely to occur together. This should be carried out using a one-tailed test, rather than the two-tailed test recommended in Robson (1969) or Seber (1982). To do this, the test statistic z is formed using equation (1) with the statistics $R.1$, $S.1$, and $R.2$, $S.2$ as defined for the type-1 loss model in Table 3(b). A significant result of this test strongly suggests that handling mortality is also present, even if it is not detected by our test. The biases from the two sources appear to be almost additive, so applying Robson's estimates allowing for type-1 loss will eliminate the bias due to this cause, but have no effect in reducing the bias due to handling of the previously marked captures. We give an example of this in the next section.

Once the data are collected and if it is apparent that handling effects occurred at one or more sample times, the full and death-only Jolly–Seber model analyses can be adjusted to reduce bias. The adjustments delete capture history information and so sacrifice precision. Moreover, they complicate the interpretation and decrease the biological usefulness of some estimates. There are several plausible procedures, which we applied to a simulated set of data generated using parameters chosen to permit comparison with the analytic results on bias given in Table 2 for a handling mortality of 67% at time $i=3$. Because the simulation is stochastic, the 'true values' (Table 5) for N_i and ϕ_i may depart slightly from the parameters used to generate the data (Arnason and Baniuk 1978, chapter 6). The standard errors (SE in Table 5) all include the variance component allowing for this stochastic deviation (Arnason and Baniuk 1978, section 5.2.1). For each procedure, estimates and standard errors are shown for the death-only model estimates of abundance and survival because the true model allowed no births. The full model estimates were also applied to these data but are not reported here because they give similar, though slightly less precise, estimates for each procedure.

When the data are analyzed without adjustment, the results are very close to the expected results (Table 2) for a 67% handling mortality rate. At this value, the power of our test at the 5% significance level is about 60% and the handling mortality at time 3 is detected. In Method 1 (Table 5), we use the modified analysis (Robson 1969; Arnason and Baniuk 1978) that permits unequal survival between marked and unmarked animals. It does not reduce the relative bias but it is less serious because precision is lost. This is because there is still heterogeneity within the marked subclass. Although the modified analysis was of no help here, it will remove all bias due to type-1 loss in the abundance estimates (Arnason and Baniuk 1978; Cormack 1972). It may thus have some applicability in real situations where type-1 loss and handling mortality are likely to occur simultaneously. The survival rate estimate of the modified full model is not useful (it estimates gross survival in the marked subset only), but the modified death-only model survival estimate is unbiased for gross survival in the total population (Arnason and Baniuk 1978, section 5.2.4). In the next section we give an example of using these modified estimates where we suspect both type-1 loss and handling mortality are occurring.

In Method 2 (Table 5), all 88 animals that were handled at time 3 were eliminated. This completely removes the source of the bias but makes the abundance estimates questionable because we do not know exactly how many of these 88 animals were present before h (full model) or after h (full and death-only models). (Note that the "true" abundances in Table 3 have been adjusted to remove any survivors of the 88 animals, but we could not do this after time 3 if these were real data.) Consequently, the abundance estimates underestimate the actual abundances. The survival estimates are all unbiased and unaffected by handling; they estimate "natural" survival only. Because time 3 is eliminated, $\hat{\phi}_2$ estimates survival from time 2 to time 4 (expected survival rate $= 0.75^2 = 0.56$).

In Method 3 (Table 5), sample time 3 is omitted (all captures at time 3 are ignored) but the capture history of each handled animal is retained at all other times. In this case, 43 of the 88 handled animals were seen only at time 3, so their histories are lost completely. This cannot remove bias completely as there is still heterogeneity of survival present between times 2 and 4 but it seems to reduce the bias to negligible levels. A simulation investigation over a wide range of parameter values showed this is generally true. Using the modified analysis here will reduce the bias further but is not worth the price of lost estimates ($\hat{N}_1$ and $\hat{\phi}_1$ are unobtainable) and reduced precision. The main advantages of Method 3 are that it is easiest to implement, and it gives a nearly unbiased estimate of the gross survival rate (including handling mortality) between times 2 and 4, which may be of more interest than natural survival in some applications. For the parameters of Table 5, the expected gross survival rate between times 2 and 4 is: $0.75 \times$ gross survival between times 3 and 4 $= 0.48$. The true value (0.50) given in Table 5 is the actual proportion that survived in this stochastic simulation. The formula for gross survival rate is given in Appendix A.

In summary, there is no way to obtain an unbiased estimate of N_h, but Method 1 (analyze the data as is, discard $\hat{N}_h$) will give the most precise, unbiased estimate of the remaining N_i. No method will give unbiased estimates of ϕ_{h-1} or ϕ_h but use Method 2 to estimate their product (natural survival) or Method 3 to estimate the gross survival rate accounting for handling mortality from $h-1$ to $h+1$. The methods can all be applied, with suitable modification, to situations with handling mortality at more than one sample time, though Method 3 is again the easiest to implement and sacrifices the least information. Although abundance estimates are lost by these methods at each time when there is a handling effect, an unbiased sequence, without gaps, of survival estimates can still be obtained.

Handling mortality can be avoided altogether by changing the recapture strategy. Band-removal methods (Brownie et al. 1978) eliminate all risk of handling or marking effects but usually provide survival estimates only. Brownie and Robson (1983, model R2) give estimates of ϕ_i and $\phi_i\theta_i$ (survival of non-handled and handled, respectively) for the Jolly–Seber full model with handling mortality. However, the estimates and their test for handling effects require, in addition to normal captures and releases at each sample time, that some animals be sighted, and if marked, their mark recorded without handling. This requirement is probably unattainable for most fish populations.

Steps should be taken to minimize the occurrence of handling mortality. A careful examination of relevant fisheries techniques in manuals (e.g. Bagenal and Tesch 1978; Nielsen and Johnson 1983) is essential. Fish should be handled carefully but quickly during sampling. Cold-water fishes should not be sampled during mid-summer unless special techniques, such as cooling the water used during sampling, are employed. Catch gear should be inspected frequently and the fish-marking technique chosen carefully. When water temperature effects are unlikely to occur, a sample of both newly marked and previously marked fish should be held in a live pen. The latter group provides a control for assessing marking mortality (Paulik and Robson 1969) and, if it suffers little mortality, indicates the absence of handling mortality. Mark–recapture statistics should be formed at intermediate points during experiments. In the example of lake whitefish in the following section, the low r_i/s_i ratios for two sampling periods, relative to ratio values at adjacent times, were the first indication of handling mortality. Epilimnetic temperatures were greater than 12°C during the periods where handling mortality was detected. In subsequent sampling, when epilimnetic temperatures were $\geqslant 12°C$, ice was used to cool water in which lake whitefish were held during sampling. We could detect no handling mortality in the mark–recapture data for sampling periods at which this method was employed.

An Example

Multiple mark–recapture experiments were conducted on lake whitefish from 1973 to 1977 in the two basins of Lake 226 (Cleugh and Hauser 1971), the Experimental Lakes Area, northwestern Ontario (Mills 1981, 1985). This small lake (16.1 ha) was divided into separate basins (NE and SW) by a vinyl sea curtain which eliminated movement of whitefish between basins. No immigration or emigration occurred in either basin from 1973 to 1977. New recruits (young-of-the-year from 1973 to 1977) were excluded from analyses so the Jolly–Seber death-only model was appropriate. Mark–recapture data for each basin were tested for the no-tag-loss assumption (Arnason and Mills 1981) and the equal catchability assumption (using the methods of Carothers 1973 and Pollock 1975). Significant tag loss was detected for 161 whitefish tagged with gun-type tags in the NE basin; these fish were excluded from subsequent analyses in this paper. No other violations of either assumption were detected.

We present only the results for the NE basin because results for both basins were very similar. The first analysis in Table 6 shows the characteristic pattern produced by handling mortality in samples 2 and 3 (May and June, 1974); the $\hat{N}_i$ at these times are greatly inflated (relative to the general downward

TABLE 6. Abundance ($\hat{N}_i$) and survival ($\hat{\phi}_i$) estimates from Jolly–Seber death-only analyses for NE basin lake whitefish (standard errors shown in parentheses). The first analysis is performed on all data without any adjustment for handling mortality, but the modified death-only estimates (second analysis) allow for type-1 loss. The third analysis removes handling bias at times 2 and 3 by omitting captures at these times. The final analysis applies Jolly's (1982) model assuming constant survival per unit time to the third analysis data.

		First analysis (Death-only)		Second analysis (Modified death-only)		Third analysis (Omit samples 2, 3)		Final analysis (constant survival)	
Sample	Sampling date	$\hat{N}_i$	$\hat{\phi}_i$	$\hat{N}_i$	$\hat{\phi}_i$	$\hat{N}_i$	$\hat{\phi}_i$	$\hat{N}_i$	$\hat{\phi}_i$
1	Sept. 1973	596 (37)	1.00[c] (0.20)	—[b]	—[b]	516 (33)	0.77[a] (0.08)	527 (28)	0.81[a] (0.02)
2	May 1974	836 (161)	1.00[c] (0.29)	408 (39)	1.00[c] (0.28)				
3	June 1974	1006 (217)	0.39 (0.09)	469 (121)	0.77 (0.23)				
4	July 1974	395 (30)	1.00[c] (0.10)	358 (40)	1.00[c] (0.16)	395 (30)	1.00[c] (0.10)	438 (17)	0.97 (0.00)
5	Sept. 1974	411 (30)	0.93 (0.10)	396 (40)	0.92 (0.14)	411 (30)	0.93 (0.10)	420 (13)	0.84 (0.02)
6	June 1975	380 (34)	0.85 (0.11)	364 (37)	0.90 (0.16)	380 (32)	0.85 (0.11)	363 (13)	0.98 (0.00)
7	July 1975	322 (33)	1.00[c] (0.34)	328 (45)	1.00[c] (0.43)	322 (32)	1.00[c] (0.34)	338 (11)	0.98 (0.00)
8	Aug. 1975	346 (113)	0.92 (0.31)	378 (153)	0.98 (0.42)	346 (113)	0.92 (0.31)	332 (14)	0.96 (0.01)
9	Sept. 1975	319 (32)	0.93 (0.13)	370 (48)	0.79 (0.14)	319 (31)	0.93 (0.13)	319 (13)	0.86 (0.02)
10	May 1976	295 (32)	0.68 (0.10)	291 (31)	0.69 (0.10)	295 (31)	0.69 (0.10)	272 (16)	0.91 (0.01)
11	Sept. 1976	202 (18)	—[b]	199 (18)	—[b]	202 (21)	—[b]	234 (18)	0.82 (0.02)
12	May–Sept. 1977	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	186 (27)	—[b]

[a] $\hat{\phi}_1$ for the last 2 analyses estimates gross survival of all fish from September, 1973 to July, 1974.

[b] Indicates estimates near the beginning and end of the experiment that are not separately identifiable for the given model.

[c] Inadmissible estimates (e.g. $\hat{\phi} > 1.0$) are reset by the analysis program to the nearest admissible value (e.g. 1.0).

TABLE 7. Results of the test for handling mortality of NE basin lake whitefish. Statistics $R.1/S.1$ and $R.2/S.2$ (Table 3(b)) are recapture rates of animals not handled and handled, respectively. The test statistic, z, is computed using equation (1). The significance of z (probability of a larger z if no handling mortality occurs at time i) is given by P. Values of $P < 0.10$, indicated by '*', are considered significant. When handling mortality is suspected at two consecutive times, the test is repeated after omitting captures at the first of the two times. This procedure gave the result shown for sample 3(a).

Sample	Sampling date	$R.1$	$S.1$	$R.2$	$S.2$	$R.1/S.1$	$R.2/S.2$	z	P
2	May 1974	75	121	15	37	0.62	0.41	2.31	0.01*
3	June 1974	14	37	14	47	0.41	0.30	1.03	0.15
3(a)	June 1974	74	121	14	47	0.61	0.30	3.65	<0.01*
4	July 1974	14	47	42	56	0.30	0.75	<0	>0.50
5	Sept. 1974	36	56	57	89	0.64	0.64	0.03	0.49
6	June 1975	51	89	47	77	0.57	0.61	<0	>0.50
7	July 1975	45	77	29	43	0.58	0.67	<0	>0.50
8	Aug. 1975	27	43	4	7	0.63	0.57	0.29	0.39
9	Sept. 1975	2	7	39	67	0.29	0.58	<0	>0.50
10	May 1976	36	67	38	77	0.54	0.49	0.53	0.30
11	Sept. 1976	15	77	32	106	0.19	0.30	<0	>0.50

trend evident in samples 1, 4, 5...) and $\hat{\phi}_1$ is thus an overestimate (>1) and $\hat{\phi}_3$ is an underestimate.

A number of tests for survival heterogeneity were applied to these data (see Seber 1982 for methods; Mills 1981 for application to these data). We tested for the following influences on survival at any or all sample times: (1) capture method (trap net or gill net); (2) tagging (type-1 loss); (3) number of previous captures; (4) age, using age-groups 1–3, 4–6, 7–10, 11–15, and 16–25 yr; (5) length, using groups 17–24, 25–29, 30–34, and 35–46 cm; and (6) sex. Significant type-1 loss was detected in sample 2 (May, 1974; $z = 3.2$, $P < 0.05$) and 3 (June, 1974; $z = 2.1$, $P < 0.05$). The only other significant result was one due to age and was evidently strongly associated with type-1 loss; the 4–6 age-group had significantly higher mortality than other age-groups in June 1974, but most of the animals in this age-group were first caught and tagged in this sample. Applying the modified death-only estimates, as shown in the second analysis (Table 6), should completely eliminate bias due to type-1 loss in both the abundance and survival estimates, although the survival estimates represent the gross survival, including unobserved losses due to tagging. Clearly, there is great improvement in the estimates at times 2 and 3. The estimates at time 1, which are biologically important, cannot be formed, and there still appears to be bias in the time 3 estimate. None of the other estimates are changed greatly by the modified analysis, apart from a slight loss in precision, confirming that the type-1 loss is probably confined to times 2 and 3. In particular, the inadmissable $\hat{\phi}_7 = \hat{N}_8/(\hat{N}_7 - n_7 + s_7)$ is probably due to poor precision in $\hat{N}_8$ and not to handling effects.

Tests for handling mortality (Table 7) indicated significant handling mortality after the May, 1974 sample but not after the June, 1974 sample. However, when the May captures were ignored and the recapture rate of fish handled in June, 1974 was compared with non-handled fish seen in September, 1973, it indicated a significant handling mortality after the June, 1974 sample as well (sample 3(a) in Table 7). It was the only significant result obtained by the lagged test (i.e. at $h+1$, omitting captures at time h). A third analysis (Table 6) was then carried out to eliminate all handling effects, including type-1 loss, by omitting all captures at times 2 and 3. This restores the estimate of the initial population size (September, 1973) and gives an unbiased estimate of the gross survival rate over the 8 months from September, 1973 to July, 1974. The estimates at and after time 4 are unchanged from those of the first analysis.

If one takes account of the unequal times between samples, the estimates from the third analysis support the hypothesis that monthly survival is constant; there were few losses-on-capture and a plot of $\log(\hat{N}_i)$ vs sample time, expressed in months elapsed since time 1, appeared to be linear. Therefore, we fitted these data using Jolly's (1982) death-only model with constant monthly survival. The model was fitted iteratively and converged quickly (2 iterations) to the estimates shown as our final analysis (Table 6). However, if this model is fitted to the original data, as used in the first analysis, the iterations fail to converge. This demonstrates the importance of taking careful steps to detect and remove sources of bias before such reduced-parameter models (Jolly 1982; Brownie et al. 1986) are applied.

It might appear that the bias in the first analysis could be solely the result of type-1 loss. There are statistical and biological reasons why this is probably not the case here. Most of the animals handled and returned at times 2 and 3 were newly tagged: 27 out of 37 returns at time 2, 41 out of 47 at time 3. Even if additional mortality was due to handling and not due to tagging alone, the Robson (1969) type-1 loss model would remove most of the bias, as occurred in our second analysis. Yet there were other sample times when large numbers of new tags were administered using identical tagging procedures (e.g. 44 new tags at time 4, 31 at time 5) that showed no evidence of type-1 loss. In any case, our methods of bias correction for handling will also remove any bias due to type-1 loss. However, it is worth determining if type-1 loss is the sole source of the bias because the Robson model can be applied even if there is type-1 loss at every sample, whereas our corrections cannot.

The cause of the handling mortality in May and June of 1974 was probably due to high temperatures of water (>12°C) in tubs used to hold fish during sampling. When fish were released after sampling, they swam normally in May, but by the end of the June sampling period some of the released whitefish were experiencing buoyancy problems, characterized by erratic circling either on the lake surface or vertically. Commencing in the July sampling period, water in fish holding tubs was cooled (<10°C) using ice. Aberrant behavior was not observed when fish were released. Ice was used during subsequent sampling periods when epilimnetic temperatures were ⩾12°C.

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Appendix A. Computing Asymptotic Relative Bias

We will only sketch the development of the relative bias formulae. The method is to develop the recapture probability (χ_i) for handled and non-handled animals, use this to derive the expected value of the statistics that appear in the Jolly–Seber formulae, and finally, substitute these expectations in the ARB formulae (as in Arnason and Mills 1981). We give the first 2 steps here (but omit the straightforward last step) for the general model with handling mortality of $1 - \theta_i$ at time i. Recall that survival from i to $i + 1$ is at rate ϕ_i for non-handled and at rate $\phi_i\theta_i$ for handled animals. Capture rate at time i is π_i and we assume no loss on capture. The complement of the recapture probabilities can be seen to satisfy the following relationships:

$$\begin{aligned} 1 - \chi_i &= P(\text{an animal is not seen after } i \mid \text{it was not seen at } i \text{ but was alive at time } i), \\ &= (1 - \phi_i) + \phi_i(1 - \pi_{i+1})\,(1 - \chi_{i+1}), \text{ and} \\ 1 - \chi_i' &= P(\text{an animal is not seen after } i \mid \text{it was seen (handled) at } i \text{ and returned}), \\ &= (1 - \phi_i\theta_i) + \phi_i\theta_i(1 - \pi_{i+1})\,(1 - \chi_{i+1}). \end{aligned}$$

Hence $\chi_i' = \theta_i\chi_i$.

Given the structure (N_i, M_i) at time i, the expectations of the following statistics are needed:

Z_i = number seen before i, not seen at i, and seen after i,
$E(Z_i) = (M_i - E(m_i))\chi_i = M_i(1 - \pi_i)\chi_i$,
Z_i' = number not seen at i and seen after i (used in death-only model),
$E(Z_i') = (N_i - E(n_i))\chi_i = N_i(1 - \pi_i)\chi_i$,
r_i = number seen at i and seen after i,
$E(r_i) = E(n_i)\chi_i' = N_i\pi_i\theta_i\chi_i$,

and we require the recursions for the structure at $i + 1$ given that at i:

$$\begin{aligned} E(M_{i+1}) &= N_i\pi_i\phi_i\theta_i + M_i(1 - \pi_i)\phi_i, \text{ and} \\ E(N_{i+1}) &= (N_i - E(n_i))\phi_i + E(n_i)\phi_i\theta_i + B_i \\ &= N_i\phi_i[1 - \pi_i(1 - \theta_i)] + B_i. \end{aligned}$$

Note that this is just $N_i\tilde{\phi}_i + B_i$ where $\tilde{\phi}_i$ is the gross survival rate $\phi_i[1 - \pi_i(1 - \theta_i)]$. It is also convenient to define

$$\begin{aligned} \xi_i &= ((1 - \pi_i)(1 - \theta_i)/\theta_i) + 1 = \pi_i + (1 - \pi_i)/\theta_i \\ &= \tilde{\phi}_i/(\phi_i\theta_i). \end{aligned}$$

Using these formulae, one can then derive expressions for the expected values of the full model estimates for the general handling effects model. The results for $E(\hat{N}_i)$ and $E(\hat{\phi}_i)$ can also be shown to hold for the death-only model estimates:

$$\begin{aligned} E(\hat{N}_i) &= N_i\xi_i, \\ E(\hat{M}_i) &= M_i\xi_i, \\ E(\hat{B}_i) &= B_i\xi_{i+1}, \text{ and} \\ E(\hat{\phi}_i) &= \phi_i\theta_i\xi_{i+1} = \tilde{\phi}_i\xi_{i+1}/\xi_i \end{aligned}$$

The last equation shows that $\hat{\phi}_i$ is an unbiased estimate of gross survival if $\pi_i = \pi_{i+1}$ and $\theta_i = \theta_{i+1}$. The ARB are easily derived from these equations and give the results reported in Table 1 for the special case of $i = h$ or h-1 and $\theta_j = 1$ for $j \neq h$.

Appendix B. Magnitude of Handling Mortality for $P_1 = P_2$

Referring to the handling mortality model in Table 3(b) for statistics definitions, we have (in expectation):

$$P_1 = E(R.1)/E(S.1).$$

Given n_{i-1} and allowing for handling mortality at all times, it may be seen, using results of Appendix A, that

$$\begin{aligned} E(R.1) &= n_{i-1}\phi_{i-1}\theta_{i-1}(1 - \pi_i)\chi_i, \text{ and} \\ E(S.1) &= n_{i-1}(1 - \theta_{i-1}\phi_{i-1}\pi_i). \end{aligned}$$

Hence $P_1 = C(i)\chi_i$,

where $C(i) = \theta_{i-1}\phi_{i-1}(1 - \pi_i)/(1 - \theta_{i-1}\phi_{i-1}\pi_i)$.

Similarly, $P_2 = E(R.2)/E(S.2)$ and, given n_i,

$$\begin{aligned} E(R.2) &= n_i\chi_i' = n_i\theta_i\chi_i, \text{ and} \\ E(S.2) &= n_i. \end{aligned}$$

Hence $P_2 = \theta_i\chi_i$.

It is easily shown that $C(i) \leqslant 1$ for all θ_{i-1}, ϕ_{i-1}, π_i so that $P_1 \leqslant P_2$ when $\theta_i = 1$ (no handling mortality at time i, the null hypothesis). $P_1 = P_2$ when $\theta_i = C(i)$. At (or below) this value of θ_i, the test at the α% significance level has a $\beta = \alpha$ (or greater) chance of rejecting the null hypothesis. A number of other tests were investigated but none was found that gave $P_1 = P_2$ when $\theta_i = 1$ and none gave $P_1 = P_2$ for smaller handling mortalities.

Whole-Lake Metal Radiotracer Movement in Fertilized Lake Basins

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Hesslein, R. H. 1987. Whole-lake metal radiotracer movement in fertilized lake basins. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 74–82.

Seven radiotracers: ^{75}Se, ^{203}Hg, ^{85}Sr, ^{134}Cs, ^{59}Fe, ^{65}Zn, and ^{60}Co were added to the separated basins of Lake 226, Experimental Lakes Area, northwestern Ontario. The basins, L226N and L226S were part of an ongoing eutrophication experiment in which C, N, and P were being added to L226N and C and N to L226S. The radiotracers moved to the sediments by sorption to settling particles and by being directly absorbed. The loss rates ranged from 3–8%·d^{-1}. Areal distribution in the sediments was largely governed by the percent sorbed to particles. Both basins exhibited seasonal anoxic hypolimnia in which redissolution of ^{59}Fe, ^{60}Co, and ^{203}Hg was great, of ^{75}Se, ^{85}Sr, and ^{65}Zn only minimal, and of ^{134}Cs negligible. The overall behavior of each of the isotopes was nearly identical in the two basins, partly due to interbasin mixing. A least squares fit of the affinity for particles and loss rates to a whole-lake model yielded a settling velocity of 0.18 m·d^{-1} and a benthic boundary layer of 370 μm thickness.

On a ajouté sept traceurs radioactifs, soit Se^{75}, Hg^{203}, Sr^{85}, Cs^{134}, Fe^{59}, Zn^{65} et Co^{60}, aux bassins séparés du lac 226, Région des lacs expérimentaux, au nord-ouest de l'Ontario. Les bassins L226N et L226S faisaient partie d'une expérience continue sur l'eutrophisation dans laquelle C, N et P ont été ajoutés au L226N et C et N au L226S. Les radiotraceurs se sont déplacés vers les sédiments par sorption aux particules qui se déposent et en étant directement absorbés. Les taux de perte ont varié de 3 à8 %·jour.$^{-1}$ La distribution superficielle dans les sédiments était en grande partie régie par le pourcentage qui s'est fixé aux particules. Dans les deux bassins, il y a eu dans l'hypolimnion une anoxie saisonnière où la redissolution de Fe^{59}, de Co^{60} et de Hg^{203} a été élevée, celle de Se^{75}, Sr^{85} et de Zn^{65} a été minimale et celle de Cs^{134} a été négligeable. Le comportement global de chacun des isotopes a été presque identique dans les deux bassins, ce qui est en partie attribuable au mélange entre les bassins. Un ajustement par la méthode des moindres carrés de l'affinité pour les particules et des taux de perte apporté au modèle établi pour l'ensemble des lacs a donné une vitesse de sédimentation de 0,18 m·jour^{-1} et une couche limite benthique de 370 μm d'épaisseur.

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Hesslein et al. (1980) reported on the geochemical results of a whole-lake radiotracer experiment in Lake 224, an oligotrophic lake in the Experimental Lakes Area, northwestern Ontario. They showed that the six isotopes added; ^{75}Se, ^{203}Hg, ^{134}Cs, ^{59}Fe, ^{65}Zn, and ^{60}Co were rapidly (40–100 d) removed to sediments. The removal rates and sediment distributions were related to morphometric characteristics of the lake and to the distribution of isotopes between particulate and dissolved phases.

A very simple model was presented to describe the removal to the sediments, but it was not process-oriented, nor did it allow for generalization between different lakes. Recently, Santschi et al. (1986) have presented a complex model for the interpretation of data from many radioisotope experiments in enclosures. This model is based on physical and chemical processes and can be applied in a general sense to lakes. The model is used here to interpret results of another whole-lake experiment (Lake 226), and to compare these results with the earlier ones from Lake 224.

The radioisotope addition to both the north and south basins of Lake 226 (L226N, L226S) on July 25, 1977 had the original purpose of testing in general the replicability of whole-lake results and to examine the effects of trophic status on the pathways of the added isotopes. The morphometric characteristics and the trophic statuses of the two basins result in seasonally anoxic hypolimnia in both. The dramatic effects of the reducing conditions on the concentrations of the radioisotopes have also been examined in this presentation.

The two basins of Lake 226 were separated by a curtain in 1973 as part of a eutrophication experiment (Schindler 1974). Carbon, nitrogen, and phosphorus were added to L226N and only carbon and nitrogen to L226S. The fertilization resulted in mean euphotic zone productivities (1973–76) of 68 and 41 g C·m^{-2}·yr,$^{-1}$, respectively (Fee 1980). The productivity of L224 (1974–76) the site of the previous radioisotope addition (Hesslein et al. 1980) had been 16 g C·m^{-2}·yr^{-1} (Fee 1980). Unfortunately, during the summer of 1977, the year of the radioisotope addition, significant leakage occurred across the curtain between L226N and L226S, as evidenced by total phosphorus concentrations (Findlay and Kasian 1986). This means that the epilimnetic aspects of the radiotracer additions cannot be considered to represent two entirely independent

TABLE 1. Morphometric and mean chemical characteristics of Lake 226N and Lake 226S (July–October 1977) and Lake 224 (July–October 1976).

	L226N	L226S	L224[a]
Area (ha)	8.33	7.77	25.9
Volume (10^5 m^3)	4.72	4.89	30.1
Epilimnion temperature (°C)	15.2	15.3	18.0
pH	7.0	7.0	6.75
Total dissolved nitrogen ($\mu g \cdot L^{-1}$)	244	256	225
Particulate nitrogen ($\mu g \cdot L^{-1}$)	232	209	30
Total dissolved phosphorus ($\mu g \cdot L^{-1}$)	6.1	5.3	2.0
Particulate phosphorus ($\mu g \cdot L^{-1}$)	16.6	13.0	3.0
Dissolved inorganic carbon ($\mu mole \cdot L^{-1}$)	136	141	75
Dissolved organic carbon ($\mu mole \cdot L^{-1}$)	553	568	285
Particulate carbon ($\mu g \cdot L^{-1}$)	1606	1430	550
Chlorophyll *a*	15.7	13.0	2.7
Sodium ($mg \cdot L^{-1}$)	1.75	1.80	0.75
Potassium ($mg \cdot L^{-1}$)	0.22	0.25	0.37
Magnesium ($mg \cdot L^{-1}$)	0.62	0.68	0.43
Calcium ($mg \cdot L^{-1}$)	1.82	1.90	1.75
Sulfate ($mg \cdot L^{-1}$)	3.4	3.4	2.6

[a] From Hesslein et al. (1980).

experiments. A rock sill separates the two basins below the epilimnia. The morphometries of the two basins of L226 are very similar (Torgersen et al. 1982) although L226N is slightly deeper (14.7 m vs. 11 m) and larger (8.33 ha vs. 7.77 ha). Both basins have areas of sandy as well as soft organically rich sediments to water depths of 4 m and almost exclusively soft organic sediments in deeper areas. More detailed descriptions of the two basins and their biota can be found in Shearer et al. 1987; Findlay and Kasian 1987; Mills and Chalanchuk 1987. A summary of chemical–physical conditions in the lakes in 1977 is given in Table 1.

Methods

The isotopes ^{75}Se, ^{203}Hg, ^{85}Sr, ^{134}Cs, ^{59}Fe, and ^{60}Co were added to both basins of L226 on July 25, 1977. All isotopes except ^{203}Hg and ^{75}Se were supplied as chloride salts in HCl. The ^{203}Hg was supplied as a nitrate salt and ^{75}Se as Na_2SeO_3. The isotopes were combined and diluted into 20 L of distilled water which was then divided into two approximately equal portions; one portion was added to each basin by slowly draining it from a towed raft as in the L224 experiment (Hesslein et al. 1980). The addition was completed within an hour. Based on previous experience (Quay 1977) and spot samples, horizontal and vertical homogeneity in the epilimnion was achieved in about 24 h. Between 4.5 and 13 GBq of each isotope were added to each basin.

Sampling and analytical methods were nearly the same as those in the L224 experiment (Hesslein et al. 1980). Water samples were taken by Van Dorn bottle at the position of maximum depth in each lake. The frequency of sampling decreased from every 1 or 2 days in the first week to once every $\approx$35 days after October 1977. The samples were filtered (0.45 μm) within a few hours of sampling. The filtrate was then passed through consecutive columns of activated charcoal (Fisher Activated Coconut Charcoal 50–200 mesh), anion exchange resin (Dowex® 1–x8) and cation exchange resin (Dowex 50® w–x12). All components were then packaged separately in 50 mm diameter disposable Petri dishes. Column eluent was frequently roto-evaporated and analysed for isotopes escaping extraction. No significant losses were noted. For the purposes of this presentation we discuss only the filterable and non-filterable portions. The non-filterable consist of the sum of the charcoal and ion exchange extractions. Values given for total concentrations are the sum of filterable and non-filterable portions.

Sediment cores were taken with 5 cm diameter acrylic tubes by diver or gravity corer. Cores were sectioned at 1 cm intervals, oven dried (80°C) and packaged in Petri dishes. Integral deposition per unit area was calculated by summing results to the depth in the core at which no activity could be detected.

Gamma spectrometry was accomplished using a GeLi crystal detector coupled to a 4000 channel analyser. Spectral analyses consisted of a peak finding routine, integration, and background subtraction. All the integrals were identified as to isotope and each corrected for radioactive decay back to the day of addition. Error estimates were made based on background and integral counts. Geometry corrections were made for the varying sample thickness in the petri dishes. Self-absorption of gamma rays by the samples was negligible.

To simplify comparison of the data between lakes and isotopes, all values have been expressed as a fraction of the added amount per unit volume of per unit area. For each isotope, concentrations in the water are expressed as the measured concentration ÷ (total amount added ÷ total lake volume). Sediment data are likewise normalized to the amount added ÷ total lake area. Perfectly uniform distribution of an isotope in the water column or over the sediment surface would result in a value of 1.00 throughout.

Results and Discussion

Isotope Loss from Water to Sediments

As expected from the results of L224 whole-lake isotope addition (Hesslein et al. 1980) and the various enclosure experiments previously performed at the Experimental Lakes Area (Schindler et al. 1980; Santschi et al. 1986) the concentrations of all seven isotopes decreased rapidly in both basins over the first 50 d aftter addition. Although Santschi

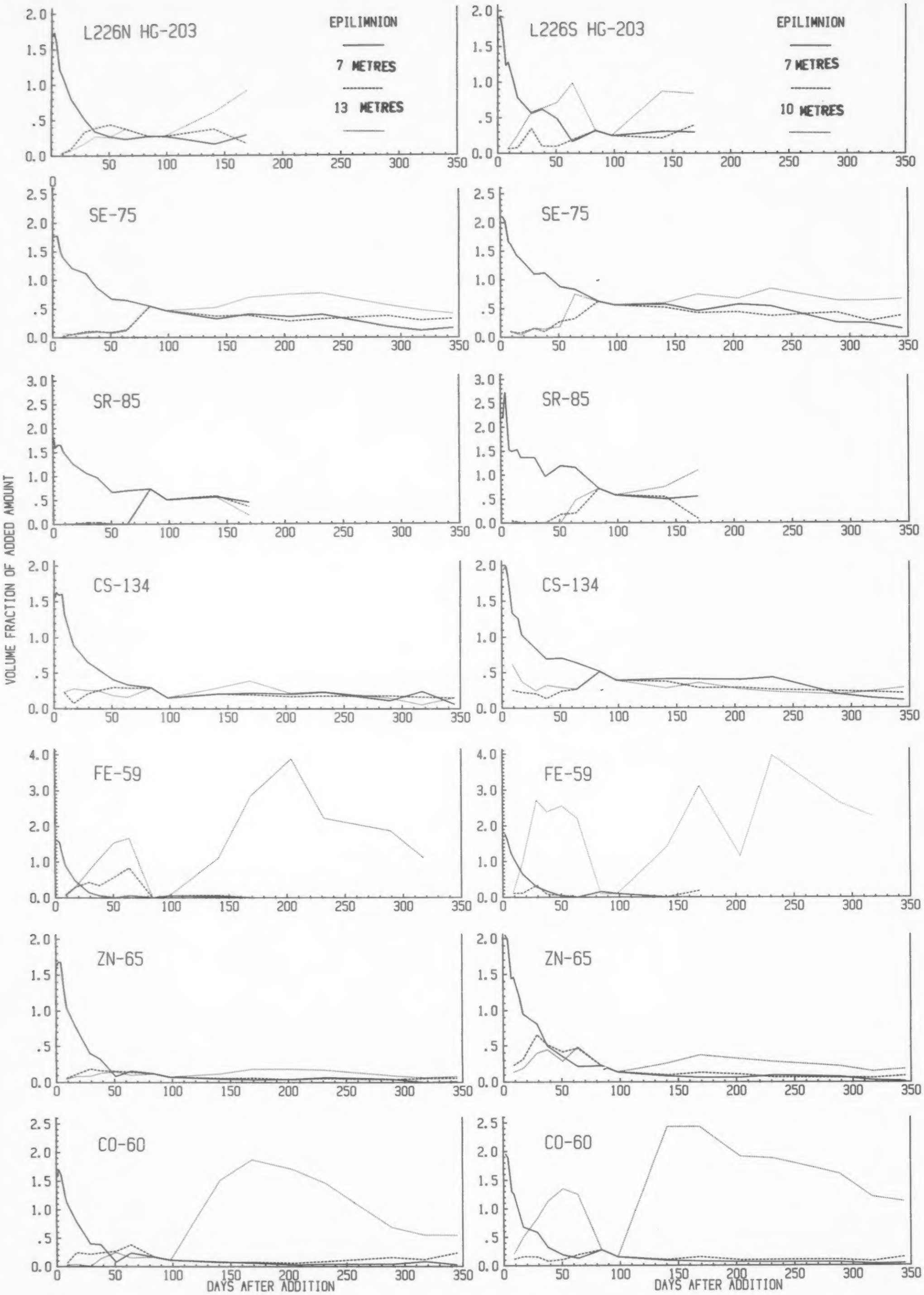

FIG. 1. Radiotracers in unfiltered water at three depths in the basins of Lake 226. Records for ^{203}Hg, ^{85}Sr, and ^{59}Fe (2 depths) end at 170 d after addition because of loss of sensitivity due to radioactive decay. The epilimnion was sampled at 1 m depth.

TABLE 2. Rate constants for isotope removal from waters, K (raw) and K_c (corrected for epilimnion deepening) and equilibrium concentration ratios Ce/Co for the epilimnia of Lakes 226N, 226S, and 224 (224 data from Hesslein et al. 1980).

	L226N			L226S			L224	
Isotope	K (d^{-1})	K_c (d^{-1})	C_e/C_o	K (d^{-1})	K_c (d^{-1})	C_e/C_o	K (d^{-1})	k_c (d^{-1})
^{75}Se	0.039	0.032	0.17	0.035	0.027	0.38	0.013	0.008
^{203}Hg	0.070	0.063	0.14	0.071	0.063	0.15	0.049	0.040
^{85}Sr	0.039	0.032	0.27	0.031	0.023	0.24		
^{134}Cs	0.049	0.042	0.11	0.050	0.042	0.20	0.025	0.016
^{59}Fe	0.081	0.074	0.00	0.071	0.063	0.00	0.043	0.039
^{65}Zn	0.060	0.053	0.02	0.042	0.034	0.04	0.028	0.019
^{60}Co	0.063	0.056	0.04	0.060	0.052	0.05	0.036	0.025

et al. (1986) presented a detailed model which will form the basis of discussion of these results, the model requires more information than was available for this experiment. I have therefore simplified some aspects of the model for this interpretation. For the determination of the initial total removal rate of isotope from water, I have used the equation (Hesslein and Slavicek 1984):

$$C_t = (C_o - C_e)\cdot e^{-\lambda_i\cdot t} - C_e$$

where C_t is the concentration at time t
C_o is the initial concentration at $t = 0$
C_e is the relatively constant concentration reached at $t = 100-200$ d after addition
λ_i is the total loss rate coefficient

In Santschi et al. (1986) the parameter C_e is not a constant but is dependent on sediment mixing and sediment sorption values.

The values for the parameters C_o and λ_i were determined by fitting the model to the data using the iterative technique of Wohlberg (1967). C_e was estimated by the mean values of concentration 100–200 d after addition (Fig. 1). After λ_i was determined, a correction was made for the dilution of concentration caused by the deepening of the epilimnia of both basins. This deepening progressed as a result of surface water cooling throughout the first 90 d of the experiment until complete fall mixing occurred. The dilution was estimated by the regression of the logarithm of the epilimnion volumes against time. The dilution rates of 0.007 d^{-1} for L226N and 0.008 d^{-1} for L226S were substracted from the raw estimates of λ_i. The raw and corrected values of λ_i and the generalized form of C_e/C_o are given in Table 2.

The total coefficient λ_i has been further broken down (Santschi et al. 1986) to rates controlled by the processes of settling particulate matter and the diffusion and sorption at the sediment–water interface:

$$\lambda_i = D\cdot A/(V\cdot z)\cdot(1-f) + S\cdot f/h$$

where D is the molecular diffusion coefficient of the metal ion in water
A is the area of sediments in contact with the epilimnion
V is the volume of the epilimnion
z is the thickness of the boundary layer at the sediment–water interface
S is the settling velocity of particles
f is the fraction of the isotope which is filterable
h is the mean depth of the epilimnion water column

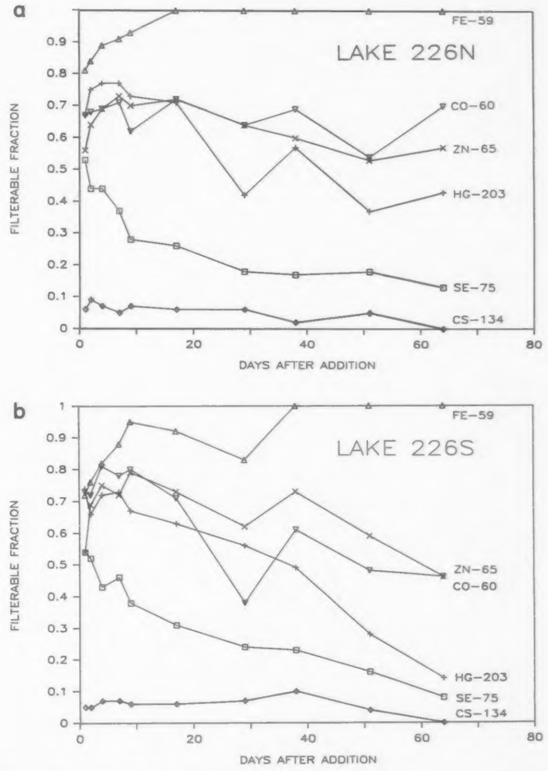

FIG. 2. Filterable fraction of radioisotopes in epilimnion samples from Lake 226N (a) and Lake 226S (b).

The dissolved metal $(1-f)$ diffuses through the boundary layer of thickness z at the coefficient of diffusion D to sorb to the sediments covering an area A. Isotopes sorbed to particles (f) settle with the characteristic velocity S over a mean distance h to escape the epilimnion.

The equation was rewritten in the linear form:

$$\lambda_i = (1/z)\cdot((D - f\cdot D)\cdot A/v) + S\cdot(f/H)$$

The data from each lake was fitted by the equation using multiple linear regression to yield estimates of the coefficients z and S. The values for the parameters A, V, and h were

TABLE 3. Areal fraction of ^{65}Zn in sediment cores at different water depths and on different dates. Values represent integrals to sediment thickness of no detectable concentration. A value of 1.000 represents deposition equal to the applied areal concentration.

Days after addition													
Depth	11	16	21	35	45	51	57	63	92	141	202	233	309
L226N Zn-65													
1	0.581			0.642				0.488					0.538
2			0.810										
3			1.067			0.567			0.952	1.247	0.322		0.960
4										0.765		1.055	
5						0.704			0.603				
6	0.071					0.682				0.447			
7	0.049						0.088			0.496			0.508
8		0.013								0.498			
9											0.626	0.300	
10													
11													
12	0.000	0.026											
13							0.390		0.392	0.635	0.106	0.510	0.577
L226S Zn-65													
1	0.311			0.815				0.545					0.876
2			0.405							0.791			
3			0.153			0.419				0.480	0.534	0.497	
4						0.523			0.846				0.818
5													
6	0.139					0.624			1.291	0.783	0.566	0.546	1.186
7	0.002												
8							0.583			0.679			
9		0.083			0.388								
10		0.060			0.247		0.475		0.663		0.508	0.683	0.684

calculated from the bathymetric data and temperature data (Cruikshank 1984). I used values for D for each isotope in the added soluble form from Li and Gregory (1974) at 18°C. Dilution corrected values of λ_i (Table 2) were used. Dilution correction for the L224 values of λ_i (Hesslein et al. 1980) were made in the same way as L226N and L226S using the temperature data of Cruikshank (1984). Values for the filterable fraction f were the means over the first 10 d of the experiment for L226N and L226S (Fig. 2) and from Fig. 5 in Hesslein et al. (1980).

The results of the regression analyses in Table 3 (SYSTAT®,for MS-DOS® personal computer operating system) showed very consistent estimates for the thickness of the boundary layer and the settling velocity. The regression coefficients are high and only the estimate of the boundary layer in L224 has a large uncertainty. No significant differences exist between lakes. The t weighted means values were 370 μm for the boundary layer and 0.19 $\text{m}\cdot\text{d}^{-1}$ for the settling velocity.

Sediment Accumulation of Isotopes

Considerable core to core variance was observed in the accumulated sediment integral even after most of the isotopes had been removed from the water (Table 4). The variance averaged 30−40% of the mean values at a given depth. This is of the same order as observed by Hesslein et al. (1980) in L224 and that described by Kelly (pers. comm., Freshwater Institute) for a whole lake ^{14}C experiment. This high variance is real and due to patterns of particle deposition and sediment sorption characteristics. Hesslein et al. (1980) showed that for the purpose of interpreting the relative deposition patterns of the different isotopes, much of the variance due to patchy deposition could be removed by using the isotope ratios. Consequently, data for all isotopes in the sediments are presented as the ratio to the concentration of ^{65}Zn (Table 4). ^{65}Zn was chosen as the denominator in this case because it was easily detected throughout the sampling period, had the most uniform distribution in the sediments, and showed little or no return to the water column in the anoxic hypolimnia. Since all results were first divided by the added quantity of isotope (see Methods), a value of 1.00 in Fig. 3a, b represents identical deposition of two isotopes relative to their addition.

Estimates of maximum and mean settling velocities were made from the accumulation of isotopes in sediments below the thermocline for comparison to velocities determined by the model fitting procedure in the previous section. The maximum velocity was determined by dividing the distance from the mixed layer to the core depth by the time at which isotopes were first detected at that depth. In L226S the first appearance in the sediments was at 6−7 m after 10 d and 10 m after 16 d while in L226N it was at 7−12 m after 11 d and 12 m after 16 d. The velocities calculated in this way all fall in the range of 0.3−0.8 $\text{m}\cdot\text{d}^{-1}$. Less than 10% of the accumulation eventually occurring at the depths used had occurred over the period of calculation. The mean settling velocities were estimated using the dates at which more than 50% of the final sediment integral had accumulated. The times to deposition at maximum depth were ≃45 d in L226S and 57 d in L226N for which mean settling velocities of 0.16 $\text{m}\cdot\text{d}^{-1}$ were calculated. These results are in good agreement with the model regression results (Table 4).

It is worth noting that the particles arriving first at the sediment had different isotope ratios than those which characterized the main sediment accumulation. Most dramatic were

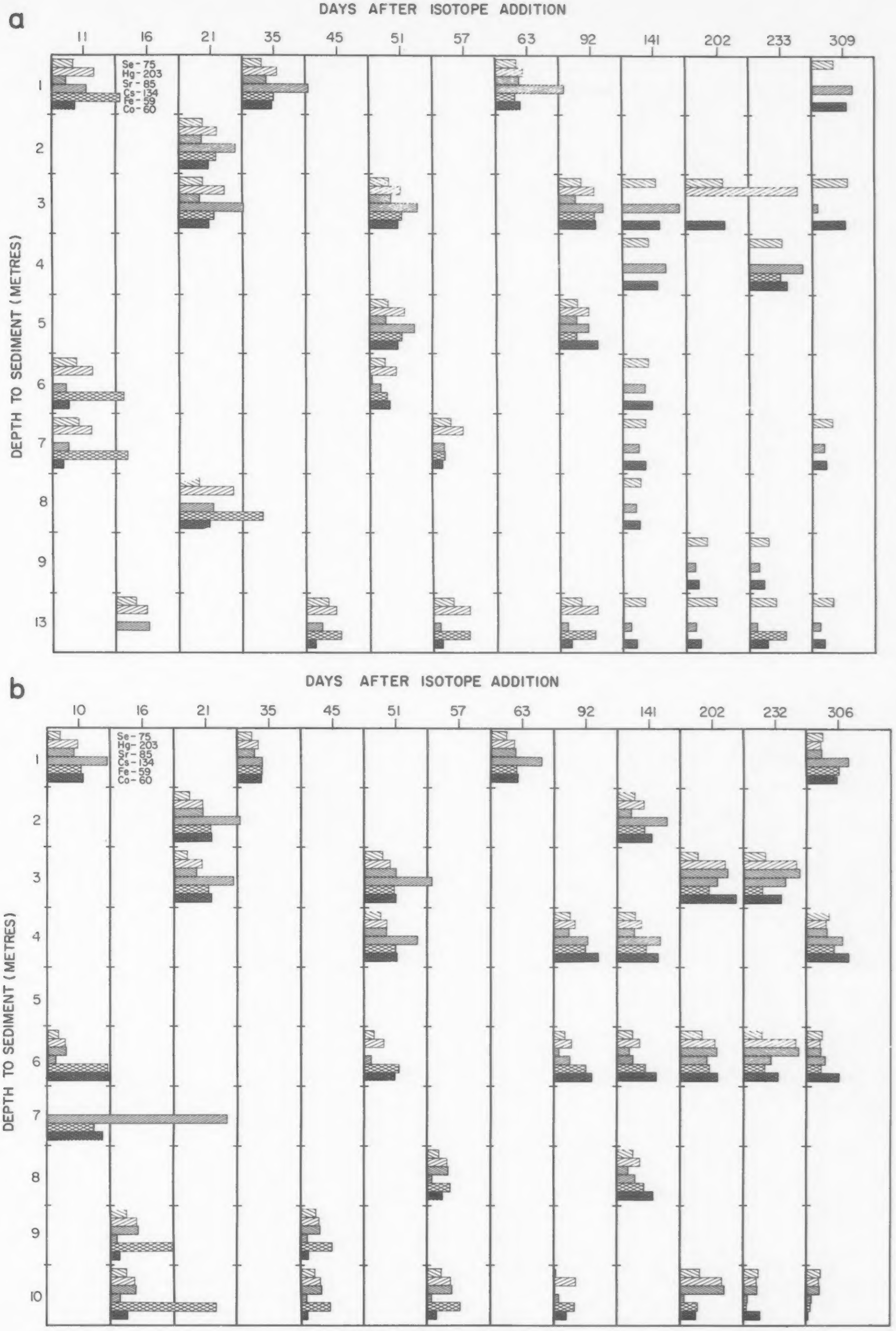

FIG. 3. The deposition of ^{75}Se, ^{203}Hg, ^{85}Sr, ^{134}Cs, ^{59}Fe, and ^{60}Co relative to that of ^{65}Zn in the sediments of Lake 226. All values represent deposition per unit area integrated with depth in the sediment cores. The raw value for each isotope was corrected for decay, divided by ^{65}Zn in the same sample, and divided by the ratio of the isotope to ^{65}Zn at addition. A value of 1.00 represents equal deposition to ^{65}Zn (see Table 3 for ^{65}Zn deposition).

TABLE 4. Results of regression analyses of Santschi et al. (1983) model of isotope loss from water columns.

	L226N	L226S	L224
n	7	7	6
r	0.657	0.854	0.636
slope	0.0317	0.0396	0.0191
intercept	0.0279	0.0309	0.0144
h (m)	10.5	8.0	17.0
h^1 (m)	3.0	2.9	5.1
D ($m^2 \cdot d^{-1}$)	10_{-4}	10^{-4}	10^{-4}
z (μm)	348	405	408
s ($m \cdot d^{-1}$)	0.177	0.204	0.171

the early Cs/Zn ratios in the deeper sediments. In the long term, ^{134}Cs was confined to sorption to epilimnetic sediments as less than 10% is filterable and subsequently settleable. This is clearly observed in the high ratio of Cs/Zn epilimnetic sediments and very low ratio in the deep water sediments. Some of the early sediment samples (10 d at 7 m in L226S and 16 d at 8 and 13 m) in L226N show much higher Cs/Zn ratios than typical of the deep samples. ^{59}Fe and ^{60}Co to a lesser extent also show higher ratios in the first few samples. It appears likely that the sorption characteristics of the rapidly settling particles are different from the particles carrying the bulk of the isotopes to the sediments. The overall pattern of deposition ratios clearly show how the less particle reactive isotopes, ^{85}Sr, ^{134}Cs, and ^{75}Se are confined to the epilimnetic sediments while ^{60}Co, ^{203}Hg, and ^{59}Fe are more uniformly distributed.

Isotopes in the Anoxic Hypolimnia

The concentrations of ^{59}Fe, ^{60}Co, and ^{203}Hg (Fig. 1) increased dramatically in the anoxic hypolimnia of L226S over the first 70 d of the experiment and again after fall overturn with the return of anoxic conditions. In L226N ^{59}Fe increased rapidly during both periods, but ^{60}Co and ^{203}Hg increased only after fall overturn. A lesser effect was observed for ^{75}Se and ^{65}Zn and no effect of anoxia was observed for ^{139}Cs and ^{85}Sr. Anoxic conditions progress from the maximum depth to 9–10 m in L226N and 8 m in L226S by the end of the period of stratification, both in summer and winter. Reoxygenation is normally complete or nearly complete in the fall due to mixing, but is seldom complete in the spring. The anoxic waters are characterized by high concentrations of reduced iron and manganese, methane, carbon dioxide and ammonia but low in sulfide (due to FeS precipitation, Cook 1981).

At the beginning of the isotope experiment anoxia was well established in the hypolimnia of the two basins. Particles destined for deep water sediments had to settle through a gradient of oxygen and Eh. Dissolution or desorption of the isotopes of Fe, Co, and Hg in the reducing environment could have occurred in the water column or at the sediment water interface after settling. It is possible that ^{60}Co and ^{203}Hg, which are more easily reduced than ^{59}Fe, desorbed from particles prior to reaching the 13 m sampling depth in L226N during the first 70 d of the experiment. After fall overturn most of the reduced compounds would be oxidized and settled and the resumption of anoxic conditions would have brought all three isotopes back into solution from the sediments.

Comparison of the concentrations of the isotopes to stable analogues in the lakes supports the above described scenario. ^{59}Fe can be compared to stable iron and although stable cobalt measurements are not available ^{60}Co can be compared to manganese which, like cobalt, is highly sensitive to redox changes. The iron and manganese were measured as part of the Experimental Lakes Area chemistry program by the methods of Stainton et al. (1977). ^{59}Fe and ^{60}Co effectively act as tracers of stable iron and manganese at two depths in L226N, 10 m and 13 m, and at 10 m depth in L226S (Fig. 4). By analogy it is expected that stable cobalt and mercury also follow the natural kind of redox cycles which have been well described for iron and manganese by Davison (1982), Davison et al. (1980, 1982), and others. Mercury has also been shown to strongly sorb to hydrous manganese oxides (Lockwood and Chen 1973). Because of the extremely low natural concentrations of cobalt and mercury in lakes ($<100\ ng \cdot L^{-1}$ and $<5\ ng \cdot L^{-1}$, respectively) the cycles of these metals have not been studied.

No obvious redox behavior can be used to explain the elevated concentrations of ^{75}Se or ^{65}Zn in the anoxic waters of the two basins. Geering et al. (1968) demonstrated the important existence of ferric selenite–ferric hydroxide complexes in soils. Iron reduction could release ^{75}Se from such a complex. It also is possible the selenium is itself reduced in anoxic waters. Zinc is not itself reducible but has been shown to associate with Mn in oceanic and freshwater manganese modules (Li 1981, 1982; Loganthan and Burau 1973). Some desorbtion may occur for ^{65}Zn due to the ion exchange competition from the much higher concentration of the divalent cations of iron and manganese. The very small increase in the concentration of ^{134}Cs at maximum depth in L226N may be due to ion exchange competition from the high ammonia concentrations present. This mechanism has been proposed previously by Evans et al. (1983).

General Discussion and Conclusions

The experimental additions of isotopes to the two basins of Lake 226 have demonstrated that the basic pathways of the isotopes ^{203}Hg, ^{75}Se, ^{85}Sr, ^{134}Cs, ^{59}Fe, ^{65}Zn, and ^{60}Co were consistent between basins and in comparison to the results of Hesslein et al. (1980) in L224. The pathways can be accurately represented using the process based model of Santschi et al. (1986). Two-fold differences in removal rates of isotopes to sediments between the L226 and L224 experiments have been explained in terms of the physical characteristics of the systems and the sorption of the isotopes to the settleable particulate material. This critical parameter of particle sorption cannot yet be predicted solely on the basis of the concentration of particles. The algal biomass was higher in both basins of L226

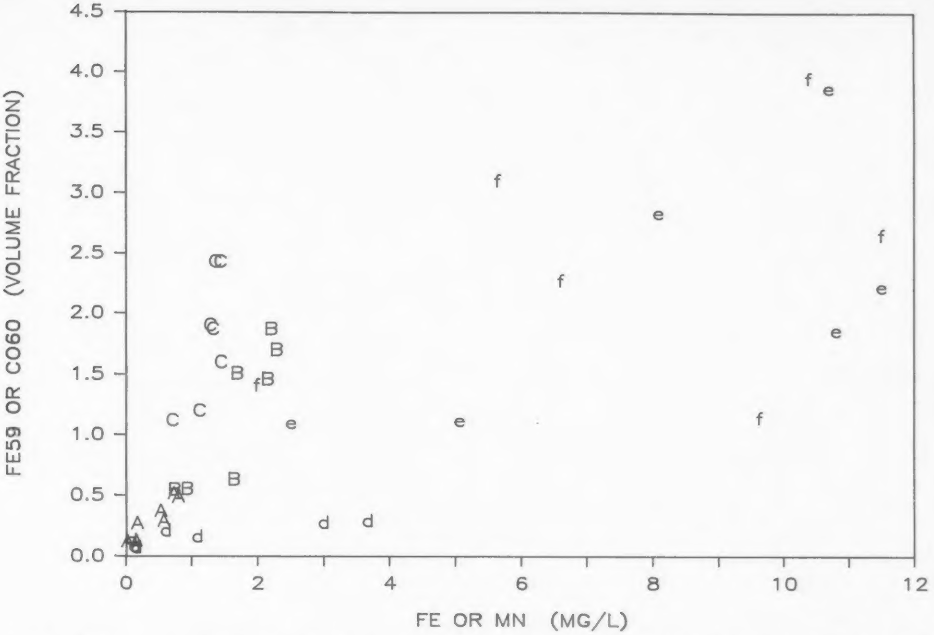

FIG. 4. ^{60}Co vs total Mn (A, B, C) and ^{59}Fe vs total Fe (d, e, f) at 10 m and 13 m depth in Lake 226N and 10 m depth in Lake 226S, respectively.

TABLE 5. Measured values for C_e, the fraction of added isotope remaining in the water after 100 and 200 d, and those calculated using sediment K_d, mixing depth, and porosity.

		K_d	C_e100 meas.	C_e100 calc.	C_e200 meas.	C_e200 calc.
L226N						
	^{75}Se	9324	0.35	0.25	0.15	0.19
	^{203}HG	900	0.20	0.77	NA	0.71
	^{85}Sr	NA[a]	0.50	NA	NA	NA
	^{134}Cs	6 660	0.20	0.31	0.15	0.25
	^{59}Fe	83 250	0.01	0.04	NA	0.03
	^{65}Zn	51 950	0.06	0.06	0.01	0.04
	^{60}Co	123 200	0.05	0.02	0.08	0.02
L226S						
	^{75}Se	9 324	0.45	0.27	0.23	0.21
	^{203}Hg	900	0.30	0.79	NA	0.73
	^{85}Sr	NA	0.50	NA	NA	NA
	^{134}Cs	6 660	0.36	0.34	0.25	0.27
	^{59}Fe	83 250	0.01	0.04	NA	0.03
	^{65}Zn	51 950	0.10	0.06	0.02	0.08
	^{60}Co	123 200	0.06	0.03	0.04	0.02

[a] NA indicates missing K_d's in calculated values or undetectable isotope due to radioactive decay.

in comparison to L224 and higher portions of the isotopes ^{203}Hg, ^{75}Se, ^{134}Cs, and ^{62}Zn were filterable, but the two isotopes with the largest filterable fraction in L224, ^{60}Co and ^{59}Fe had lower filterable fractions in L226. Mean settling velocities determined for the particles in this work were 15–20 cm·d^{-1}. These values are in good agreement with those determined in enclosures by Santschi et al. (1986) of 12–40 cm·d^{-1}. Some particles do settle more rapidly (30–80 cm·d^{-1}) and have different assemblages of sorbed isotopes. The boundary layer thickness controlling diffusion to the sediments determined in this study, 370 μm, is on the low end of the values determined by Santschi et al. (1986) in enclosure studies. Higher turbulence at the sediment–water interface in whole lakes may explain this difference.

The reducing conditions in the hypolimnia of the two L226 basins resulted in considerable desorbtion of ^{59}Fe, ^{60}Co, and ^{203}Hg from the settling particles and sediments. ^{59}Fe acted like its stable analogue and the behavior of ^{60}Co was well represented by that of stable manganese. ^{203}Hg acted more like manganese than iron. Other isotopes which respond less to the anoxic conditions could have been influenced by reducing conditions directly, or by ion exchange competition from iron, manganese, and ammonia. Long-term sediment burial of these isotopes will be slowed by the desorption process in the hypolimnia. Enhanced transport of these isotopes is likely within all the sediments, which even in well oxygenated waters are anoxic more than a few millimeters from the sediment–water interface. The redox sensitive isotopes should have a tendency to concentrate in the thin layer of oxygenated sediments. This response to reducing conditions could also periodically influence the concentrations in the water in lakes with seasonally anoxic hypolimnia. The higher concentrations maintained at the sediment–water interface could increase the concentrations in the water in the long term.

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Phytoplankton Primary Production and Light Attenuation Responses to the Experimental Acidification of a Small Canadian Shield Lake

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Phytoplankton primary production and light attenuation were monitored over a 10-yr period in Lake 223, a small, softwater, shield, lake. After 2 yr with no treatment, the lake was treated for 8 yr with sulfuric acid to decrease the epilimnetic pH from about 6.7 to 5.0. Primary production, integrated over time and depth, varied considerably during the 2 pre-treatment years. However, it increased steadily during the first 6 yr of treatment, with a total increase of more than 250%. This production increase was coincident with a decrease in epilimnetic light attenuation. After the 6th year of treatment, the production of the lake decreased although the pH level was held relatively constant during this period. Nearby control lakes tended to show a similar, though less distinct, pattern during this 10-yr period. Thus, it is difficult to separate the effects of acidification from long-term natural variation. However, there was no apparent reduction in community phytoplankton production as a consequence of the acidification and the hypothesis that acidification causes oligotrophication was not supported.

La production phytoplactonique primaire et l'atténuation lumineuse dans un petit lac à eau douce du bouclier canadien, le lac 223, ont été contrôlées au cours d'une période de 10 années. Après 2 ans, le lac a fait l'objet, pendant 8 ans, d'une addition d'acide sulfurique afin d'abaisser de 6,7 à 5,0 environ le pH épilimnétique. La production primaire, intégrée en fonction du temps et de la profondeur, a varié de façon considérable au cours des 2 années précédant le traitement. Elle a ensuite augmenté de façon constante pendant les 6 premières années du traitement; l'augmentation totale était supérieure à 250 %. Cet accroissement de la production coïncidait avec une baisse de l'atténuation lumineuse dans l'épilimnion. Après ces six années, la production a décliné bien que le pH soit demeuré relativement constant. Une évolution semblable, bien que moins nette, a été notée dans les lacs témoins au cours de ces 10 années. Il est donc difficile d'isoler les effets de l'acidification de ceux de la variation naturelle à long terme. Il n'y a cependant pas eu réduction apparente de la production phytoplanctonique suite à l'acidification et l'hypothèse que l'acidification soit cause d'oligotrophisation n'est donc pas appuyée.

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One conventional hypothesis states that as lakes are acidified, they become more oligotrophic. This hypothesis originated primarily from the Scandinavian studies of acidified lakes (Grahn and Hultberg 1974; Grahn et al. 1974; Hendrey et al. 1976; Leivestad et al. 1976; Andersson et al. 1980) and is based, in part, on the observation that acidic lakes are often very transparent. As Schindler (1980) has noted, the evidence for this oligotrophication hypothesis is largely circumstantial. Few data were obtained from these Scandinavian lakes before acidification had reached an advanced state.

When the first Experimental Lakes Area (ELA) whole lake acidification experiment was undertaken in Lake 223 (L223), it offered an ideal opportunity to test the oligotrophication hypothesis. A 10-yr record of phytoplanktonic productivity in L223 permitted the testing of this hypothesis for mean pH values greater than 5.0. A data set of light attenuation measurements, obtained in conjunction with the productivity estimates, enabled the role of light attenuation as a factor influencing production rates to be examined.

L223 is a small, dimictic lake (surface area = 27.3 ha, mean depth = 7.15 m) lying on granitic bedrock. Schindler (1980) and Schindler et al. (1980) have described the study lake. Other published papers have dealt with various early chemical and biological effects of the whole-lake acidification study (Schindler et al. 1980; Malley 1980; Muller 1980; Schindler and Turner 1982; Nero and Schindler 1983; Mills 1984). Schindler et al. (1985) provided a comprehensive summary of the overall experiment. Papers elsewhere in this volume (France 1987; Mills et al. 1987) describe in detail other changes which occurred during the study and complement the findings reported here.

During the pre-acidification years (1974 and 1975), epilimnetic pH values in L223 ranged between 6.5 and 7.0. Addition of acid (H_2SO_4) to the epilimnion began in May, 1976. Alkalinity was greatly reduced that summer (Schindler et al. 1980), although the mean pH remained near pre-acidification levels. From 1977 through 1981 enough acid was added to gradually lower the mean epilimnetic pH to 5.0 (Table 1). During 1982 and 1983, the epilimnetic pH was maintained

TABLE 1. Summary of phytoplankton productivity, surface insolation, and eplimnion pH and light attenuation for Lake 223 during the ice-free season, 1974 through 1983.

Year	Production season (days)	Mean I_o ($Ein\cdot m^{-2}\cdot d^{-1}$)	Mean EPI pH	Min EPI atten (K_d) (m^{-1})	Mean euphotic production ($mg\ C\cdot m^{-3}\cdot d^{-1}$)	Mean euphotic production ($mg\ C\cdot m^{-2}\cdot d^{-1}$)	Epilimnetic production ($mg\ C\cdot m^{-2}\cdot d^{-1}$)
1974	181	34.9	6.71	0.44	26.1	188	89
1975	190	35.8	6.65	0.40	15.9	105	78
1976	173	40.0	6.49	0.38	23.1	155	107
1977	204	32.0	6.13	0.25	27.5	196	142
1978	191	33.9	5.93	0.31	30.1	212	136
1979	186	33.8	5.64	0.24	42.0	301	173
1980	185	35.8	5.59	0.23	47.4	337	192
1981	210	35.5	5.02	0.22	60.0	429	333
1982	193	33.7	5.09	0.26	40.5	289	189
1983	199	36.2	5.13	0.26	30.7	220	187
Mean	191	35.2	5.84	0.30	34.3	243	163
SD	10	2.0	0.61	0.08	12.4	91	69

near this level. A more detailed description of the acidification procedures and pH responses is provided by Cruikshank (1984a).

Several lakes (L224, L239, L240, L382) were used as references for this study. They range from 26 to 56 ha in surface area and from 13 to 30 m in maximum depth. All stratify and are located within a 5 km radius of L223. These lakes were subject only to natural perturbations during the course of the study.

Snow cover and low solar angles prevent significant light from entering the water columns of ELA lakes under ice cover. Early studies (Fee and Shearer, unpublished data) showed that winter production was negligible (<10%) when compared to summer production in these lakes. No attempt was made to include winter estimates in this paper. "Annual" values thus represent only the ice-free season production rates (approximately May through early November).

Materials and Methods

Phytoplankton production was measured, both in L223 and in the untreated reference lakes, using an incubator–numerical model technique (Fee 1973, 1980, 1984; Shearer et al. 1985). Measurements in L223 were conducted at 1–4 wk intervals during each ice-free season. Vertical attenuation of photosynthetically active radiation (PAR) was measured at similar time intervals. Sampling and measurement procedures for the reference lakes were identical to those used for L223, although frequency and periods of sampling varied from lake to lake. Only L239 was studied in each of the 10 yr. Surface irradiance (insolation) values of PAR were continuously recorded during each ice-free season. These three data sets then were combined in the numerical model to provide production estimates integrated over depth and time ("integral production") for the ice-free portion of each year.

Water samples for incubation were collected before noon. Until August, 1974, an opaque Van Dorn sampler was used to sample discrete lake depths and these water samples were returned to the laboratory in opaque, insulated polypropylene bottles. The discovery of large variations in chlorophyll concentration with depth below the mixed layer led to the development of an integrating sampler (Fee 1976; Shearer 1978) which was used exclusively from August, 1974. Samples integrated over depth were obtained from each of the thermal zones (epilimnion, metalimnion and hypolimnion) within the euphotic zone ($I_z > 0.5\%\ I_o$) and returned to the laboratory in opaque, insulated glass or polycarbonate bottles.

The samples were partitioned under reduced light ($<5\ \mu Ein\cdot m^{-2}\cdot s^{-1}$) into Pyrex reagent bottles, injected with carbon-14 (^{14}C) and incubated in duplicate at each of 4 or 5 irradiance levels (simulating the range of in situ values) and at in situ temperatures for 2–4 h. Aliquots of each sample were retained for algal identification and counting, for chlorophyll *a* determination and for dissolved inorganic carbon (DIC) determination. The carbon-14 stock solution (activity $3.0-6.0 \times 10^5\ bq\cdot mL^{-1}$) was prepared by dilution of $Na_2{}^{14}CO_3$ (Atomic Energy of Canada Limited, Commercial Products Division) in deionized, distilled lake water. The small volumes of this stock solution added to each incubation bottle produced final radiocarbon activities of approximately $2.4-3.0 \times 10^3\ bq\cdot mL^{-1}$. Final activities were determined by direct measurement using liquid scintillation. Spot testing showed that neither the DIC concentrations nor the pH values of the samples were significantly altered by these additions.

As the pH of the lake was reduced, midsummer eplimnetic DIC concentrations often dropped to less than 20 $\mu mol\cdot L^{-1}$. Incubation times were kept short (2–3 h) whenever possible. In addition, extra DIC (as $NaHCO_3$ or H_2CO_3) was occasionally added to incubation bottles to test for carbon limitation of production.

Following incubation, each subsample was processed using acidification and bubbling techniques (Schindler et al. 1972; Wessels and Birnbaum 1979). Radiocarbon uptake was determined using liquid scintillation spectrometry. Carbon uptake in the dark was typically from 5 to 15% of the uptake measured at saturating irradiance levels and was subtracted from all production estimates.

All irradiance measurements (in-lake, lake surface, and incubator) were made with quantum sensors which measure PAR (400–700 nm). Vertical attenuation profiles were obtained in L223 and in reference lakes at approximately 2-wk intervals using cosine-corrected quantum sensors (Li-Cor 192S). Measurements were obtained at 0.5 m, at 1.0 m, and at full metre intervals thereafter (Shearer et al. 1985). Vertical attenuation coefficients (K_d, m^{-1}) of downwelling PAR were determined according to the Lambert–Bouguer Law and oper-

TABLE 2. Summary of methodological procedures which changed during the time course of this study. (See Shearer et al. 1985 for details.)

		Incubator irradiances						
Year	Sampling technique	Lighting source	Irrad. levels	Sensor type	Acidific. & bubbling	LS counter quench corr.	Integral model used	Comments
1974	discrete/range	tung–halogen	4 + dark	flat	Allihn–press	Picker–ESCR	Fee (1977)	Sampling changed in August
1975	depth range	tung–halogen	4 + dark	flat	Allihn–press	Picker–ESCR	Fee(1977)	
1976	depth range	tung–halogen	4 + dark	flat	Allihn–press	Picker–ESCR	1980 revision	See Fee (1980) for explanation
1977	depth range	tung–halogen	4 + dark	flat	Allihn–press	Picker–ESCR	1980 revision	
1978	depth range	tung–halogen	4 + dark	flat/spher	Allihn–press	Picker–ESCR	1980 revision	Irradiance sensors intercalibrated
1979	depth range	tung–halogen	4 + dark	spherical	Allihn–press	Picker–ESCR	1980 revision	
1980	depth range	tung–halogen	4 + dark	spherical	Allihn–press	Beckman–H#	1980 revision	
1981	depth range	tung–halogen	4 + dark	spherical	vac chamber	Beckman–H#	1980 revision	
1982	depth range	metal halide	5 + dark	spherical	vac chamber	Beckman–H#	Fee (1984)	New incubator design
1983	depth range	metal halide	5 + dark	spherical	vac chamber	Beckman–H#	Fee (1984)	

NOTES: Irrad. levels = number of irradiance levels at which bottles were incubated
Acidific. & bubbling = method employed for removal of inorganic carbon following incubations
Discrete = sampled at discrete depths
Depth range = sample integrated over a depth range
Tung–halogen = tungsten–halogen lamps
Flat = cosine-corrected flat plate sensor
Spherical = scalar sensor
Allihn-press = Allihn tubes with pressurized bubbling (Schindler et al. 1972)
Vac chamber = vacuum chamber bubbling (Wessels and Birnbaum 1979)
ESCR = external standards channels ratio quench correction
H# = H-number quench correction

ationally calculated as the slopes of the linear regression analyses of the natural logarithms of downwelling PAR on depth. A similar sensor (Li-Cor 190S) continuously recorded the solar irradiance at the lake surface, to which the attenuation measurements were referenced.

Although the basic method for measuring productivity was consistent during the 10-yr study period, procedural changes and refinements inevitably occurred (Table 2). Several of these changes complicate the direct comparison of data collected in different years.

Flat plate quantum sensors were used in the incubator until 1978 and spherical sensors (Li-Cor 193S, Biospherical QL100 and QSP200) thereafter (Table 2). Following a comparison of the responses of flat plate and scalar sensors in the incubator, the 1974–78 incubator data were corrected accordingly (see DeClercq and Shearer 1980 for details).

In 1982, a newly designed incubator was introduced. The earlier incubator employed multiple quartz–halogen incandescent lights. The later model used a metal halide lamp. Replicate samples run on both incubators over a 6-wk period (May and June, 1982) showed that mean, daily, euphotic integral values for L223 were 22 ± 19% lower when estimated with the new unit. Most of this discrepancy can probably be attributed to the different spectral quality of the two light sources. The metal halide lamp produced a white light with a spectral peak at approximately 580 nm. It produced little of the red light which dominated in the spectrum of the incandescent lamps. To facilitate year-to-year comparisons, all estimates based on data obtained from this new incubator have been adjusted upward by 22%.

Originally, productivity data for the early years of the study were calculated using a version of the model (Fee 1977) which incorporated factors for diurnal variation, surface inhibition, and hypolimnetic light quality. An experimental whole-lake radiocarbon addition to Lake 226 in 1978 (Bower 1981) led to the development of a revised model in 1980. This new model was used to recalculate results for the years 1976 through 1979; the revised annual values for these years averaged 21 ± 9% higher than those given in Fee (1979) and Schindler (1980). However, the input data for 1974 and 1975 were not totally compatible with the new model (Fee et al. 1982) and data from these years were not recalculated. The original 1974 and 1975 production estimates have been increased by 21% to make them comparable with the data from the other years.

For the 1983 data set, a new model (Fee 1984) was employed to calculate annual integral production values. The new model simplifies data input and can be run on microcomputers. Output from this model was extensively compared to output from the earlier model. Both models produced essentially the same annual results (± 5%).

Despite the methodological changes summarized in Table 2, we are confident that long-term trends apparent in the data (set) are real. The data for all reference basins were obtained using the same methods as for L223. Comparisons among different lakes for any given year, and among years for any given lake, are valid.

Production estimates, integrated over depth and time for each year, have been expressed as mean daily production for the ice-free season. This approach reduces the influence of unequal open-water periods in various years. All values were calculated for the euphotic zone of the lake and were corrected to account for basin morphometry (Fee 1980).

Results

During periods of low DIC concentrations, carbon added experimentally to the incubation bottles never enhanced production in the L223 experiments. Rather, when DIC concentrations in the bottles were increased by more than 100%, they often inhibited uptake rates, probably because of pH increases caused by the added bicarbonate. Carbon limitation of production apparently was not important in this lake and our radiocarbon, bottle incubations were not overestimating phytoplankton production when DIC was low.

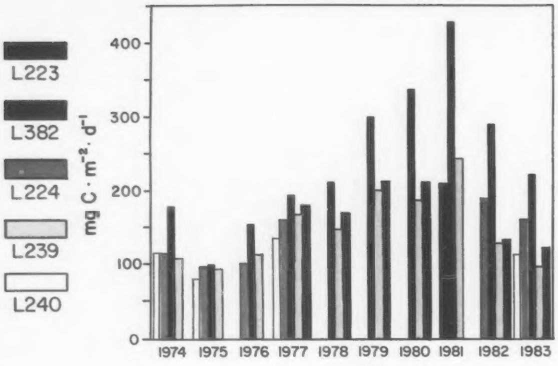


FIG. 1. Mean, ice-free phytoplankton production rates (mg C·m^{-2} d^{-1}) for the euphotic zone of Lake 223 and for four reference lakes, 1974 through 1983.

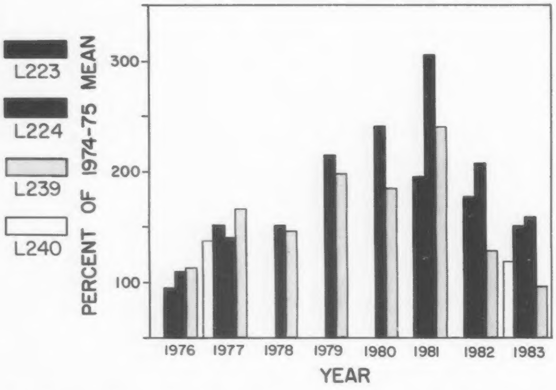


FIG. 2. Annual ice-free phytoplankton production for Lake 223 and three reference lakes (1976 through 1983) relative to the 1974–75 mean for each lake. All four lakes were monitored in 1974 and 1975, but not in every year thereafter.

Phytoplankton production, integrated over depth and time, increased continually in L223 during the first 6 yr of acidification (Table 1, Fig. 1), as the epilimnetic pH was lowered to a mean of 5.02. The integral production in the sixth year of acidification was approximately 300% greater than the mean estimate for the 2 background years (Fig. 2). Although production per unit area declined during two additional years at pH values near 5.1, it remained considerably higher than in the pre-acidification years. There was a significant negative correlation ($r = -0.79$, $n = 10$, $P < 0.001$) between mean epilimnetic production and mean epilimnetic pH for the entire 10-yr period.

The trends present in L223 also were evident in ELA reference lakes (Fig. 1 and 2). It was not clear whether the responses observed in L223 were caused by the acid or were indicative of some general phenomenon operating on all lakes in the region.

To examine changes with time in the absence of treatment, all available production data for four non-acidified lakes (Lakes 224, 239, 240, and 382) were examined. Annual mean production rates varied from lake to lake, but these rates were normalized to permit a graphical comparison (Fig. 3) of L223 production and mean reference lake production over the study period. While the epilimnetic pH in L223 was still above 6.0, there was no clear trend in L223 production relative to these reference lakes. As pH declinded from 6.13 to 5.64, production in L223 increased relative to the mean of the reference lakes. In those years when the mean pH was lowered to and held near 5.1, the production in L223 remained more constant relative to the reference lakes.

In stratified ELA lakes, there is a highly significant relationship between epilimnetic phytoplankton production and the

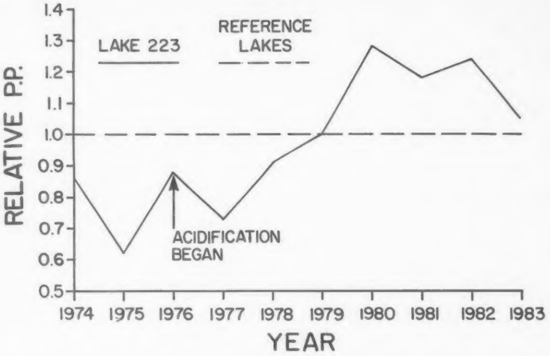


FIG. 3. Lake 223 production trends relative to the normalized mean for pooled reference lakes (L224, L239, L240, L382), 1974 through 1983. Production estimates for each lake in each year were converted to decimal fractions of the long-term means for the respective lakes and an average value for the pooled reference lakes was calculated for each year. Each annual fraction for L223 (and for the pooled reference lakes) then was divided by the pooled reference lake fraction for the same year to produce annual ratios. The reference lake means were thereby normalized to 1.0 for all years.

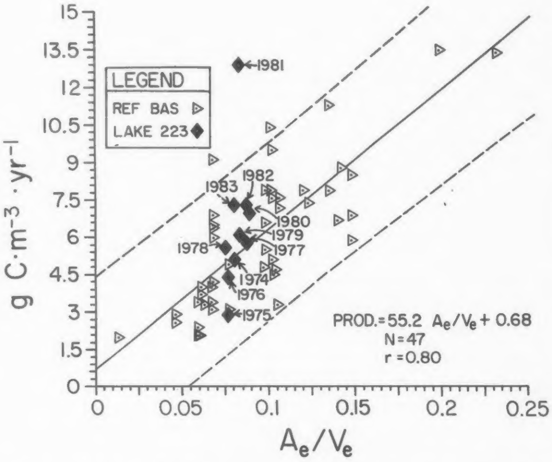


FIG. 4. Relation of epilimnetic production to $A_e : V_e$ (see text) in thermally stratified ELA lakes, 1974 through 1983. The regression line applies only to unperturbed reference lakes (REF BAS). Dashed lines are 95% confidence limits for an individual point. The L223 values (LAKE 223) are superimposed.

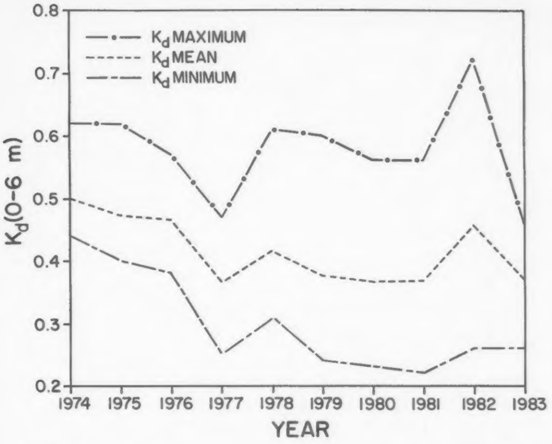


FIG. 5. Trends in the maximum, mean and minimum recorded values of the vertical attenuation coefficients (K_d) for downwelling PAR in Lake 223, 0–6 m, during each ice-free season, 1974 through 1983.

ratio of epilimnetic sediment area to epilimnetic volume (A_e: V_e) (Fee 1979). For 9 of the 10 study years, the L223 production values lie well within the expected range for non-manipulated ELA lakes of similar morphometry (Fig. 4). Only the 1981 value is higher than expected. None of the values from the treatment years lies below the expected range.

Large, summer populations of algae were common below the mixed layer of L223, particularly during the 4th and 5th yr of acidification (epilimnetic pH 5.64 and 5.59) when less than 60% of euphotic production occurred in the epilimnion (Table 1). pH values below the mixed layer ranged between 5.6 and 6.4 during this period (Linsey and Braund 1984). However, in year six of treatment (pH 5.02), when epilimnetic production exceeded the expected level (Fig. 4) for untreated ELA lakes, a summer bloom of Peridiniae in the epilimnion (Findlay 1984) boosted the epilimnetic contribution to total production to almost 80%.

The light attenuation measurements made in conjunction with the productivity studies also showed trends over time. During the first few years of acidification, the mean attenuation coefficient of downwelling PAR, K_d, decreased in the upper 6.0 m of L223 (Fig. 5). In particular, the annual minimum value of K_d decreased during the years when the pH was decreasing and then remained low. Much of this decrease occurred in 1977, when the mean epilimnetic pH showed its first significant decrease, from approximately 6.5 to 6.1. On average, more photosynthetically active radiation was available throughout much of the water column during the treatment years and particularly after the pH decreased to 6.1 and below. However, there were periods most years, particularly in the autumn, when light attenuation increased to pre-acidification levels, (Fig. 5). Thus, the annual range of K_d increased as the pH decreased. Using the values of K_d for 0 to 6 m in L223, the annual minimum value of K_d was highly correlated with annual mean pH ($r = 0.86, n = 10, P < 0.001$). Annual mean K_d and mean pH were also significantly correlated ($r = 0.66, n = 10, P < 0.05$). There was no correlation between maximum K and mean pH ($r = 0.08, n = 10, P >> 0.10$).

The attenuation of downwelling PAR also increased in non-acidified ELA lakes during the study period (Fig. 6), although the rates of increase were somewhat less than in L223. The annual range of fluctuation in attenuation of PAR was much greater in L223 during the treatment years than in any of the non-treated lakes.

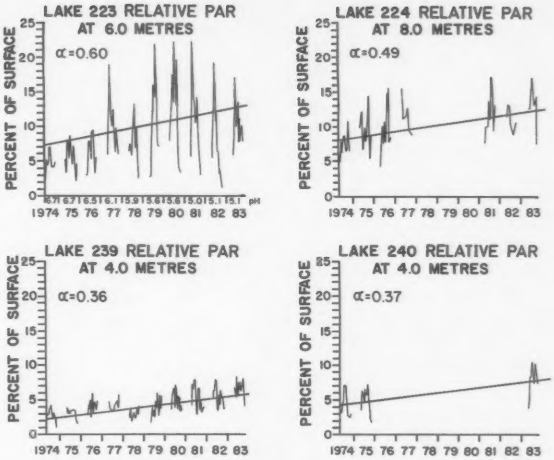


FIG. 6. Relative changes in PAR penetrating the mixed layers in Lake 223 and several reference lakes over the course of the study. Relative PAR is expressed as the percentage of surface irradiance (I_o) measured at a selected depth near the bottom of the mid-summer mixed layer. The straight line drawn through each data set represents the long-term trend of the data. Alpha (α) is the calculated slope of that trend. Note that Lake 224 was not sampled from 1978 through 1980. Lake 240 was not sampled from 1976 through 1982.

Discussion

Several factors probably contributed to the 1975–81 phytoplankton productivity increase in ELA lakes. There was a general increase in available PAR (decreased K_d) within the water column during these years (Fig. 6), perhaps because of reduced allochthonous inputs of humic matter. This period included three years (1976, 1979, 1980) when annual precipitation totals were from 19 to 28% below the long term mean (Beaty 1981, 1984) and only in 1977 was precipitation above this mean. Consequently, water renewal times were increased and nutrient cycling was probably altered because of decreased inflows and outflows. This was also a period of increased epilimnetic depths (Cruikshank 1984b), probably because of decreases in K_d and increased wind energy inputs. Decreases in K_d reduced density gradients in the metalimnion (Effler and Field 1983), thereby increasing vertical cycling of nutrients. The deepened epilimnion and greater wind inputs also may have permitted increased rates of nutrient cycling between the epilimnetic water and sediments (Fee 1979) (Fig. 4).

Because the whole-lake productivity increase in L223 during treatment years three through five was greater than in the reference lakes (Fig. 3), production in this lake apparently was responding not only to these regional factors, but also to some time-dependent factor specific to the basin, presumably the acid. The exact nature of this acidification response is unknown, but it was probably indirect and the evidence suggests a link to the reduced light attenuation.

This reduced attenuation of light in L223 can be attributed, at least in part, to the effects of acidification. Cruikshank (1984b) has documented an increase in mean secchi depth during the early stages of L223 acidification. Yan (1983) found a negative correlation between secchi depth and pH in acidified Lohi Lake, near Sudbury, Ontario, and other authors (Almer et al. 1974; Kwiatkowski and Roff 1976) have reported increased transparency with declining pH. In the south basin of L302, another ELA lake basin receiving epilimnetic additions of sulphuric acid, the attenuation of PAR decreased noticeably as the epilimnetic pH was lowered from 6.8 to near 5.6 over a 2-yr span (Shearer and DeBruyn 1986). Also, the increased fluctuations in light attenuation in L223 during the treatment years are indicative of the instability often exhibited by perturbed systems. This is a further indication that much of this attenuation decrease was acid induced. While ELA reference lakes underwent summer attenuation decreases, none of these decreases was nearly as great as that in L223.

The lowered pH of L223 may have been responsible for removing humic matter and colour from the surface waters, thus reducing the light attenuation. Unfortunately, the precise nature of the colour changes occurring in L223 during the first years of acidification was not well documented. Dickson (1978) has related the reduction of humic matter in acidified lakes to precipitation of humics because of increased aluminum levels. Effler et al. (1985) showed that attenuation of light in an acidic lake was regulated largely by "gelbstoff" (dissolved yellow organic matter) and suggested that removal of this matter by aluminum may have contributed to increased water clarity. The aluminum concentration in the epilimnion of L223 increased several fold as the pH was lowered (Schindler and Turner 1982; Schindler et al. 1987) and mean Al concentrations were negatively correlated with mean epilimnetic pH from 1976 to 1981 ($r = -0.794$, $n = 6$, $P < 0.05$). However, typical aluminum concentrations (20–60 $\mu g \cdot L^{-1}$) in L223 during acidification were much lower than those reported in the Dickson (1978) or the Effler et al. (1985) studies. Also, dissolved organic carbon (DOC) concentrations did not change significantly during these years (ELA staff, unpublished data) and were not correlated with epilimnetic pH.

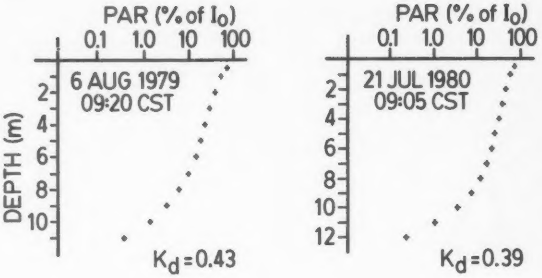
Whatever the cause of the reduced light attenuation in L223, increased PAR became available to the phytoplankton during the summer months and apparently boosted production rates in the deep-dwelling populations growing at subsaturating irradiances. Our incubations typically showed (Fig. 7) that production by these algae was light-limited. In autumn, as the mixed layer deepened, these deep, algal populations were mixed into the surface waters where they contributed both to the increased light attenuation (Fig. 4) and to increased epilimnetic production. This also explains why annual maximum K_d values (Fig. 5) showed no clear trend over time, although the minimum K_d values declined with lowered pH.

Several other theories may help to explain why phytoplankton productivity in L223 was not adversely affected and why "oligotrophication" did not occur.

The role of humic matter as a regulator of primary production has been discussed by various authors (Shapiro 1966; Sakamoto 1971; Jackson and Hecky 1980). By removing humic matter from the water column of L223, the acid may have increased the availability of iron and other trace elements, thus enhancing algal productivity.

Phosphorus–aluminum interactions should also be examined. Dickson (1978) hypothesized that phosphorus was removed from acidic waters by precipitation with aluminum. Nalewajko and Paul (1985) reported depression of both phosphate uptake and photosynthesis at aluminum concentrations of 50 $\mu g \cdot L^{-1}$ and above, and pH values of 5.2 and above. Aluminum concentrations greater than 50 $\mu g \cdot L^{-1}$ were occasionally measured in the epilimnion of L223, particularly in 1978, 1981, and 1982, but phosphorus uptake rates were not measured. Phosphorus concentrations did not decrease in L223 (Linsey and Braund 1984; Schindler et al. 1985), perhaps because aluminum concentrations remained relatively low.

(a) PAR vs. depth in Lake 223



(b) Carbon uptake vs. PAR in incubator

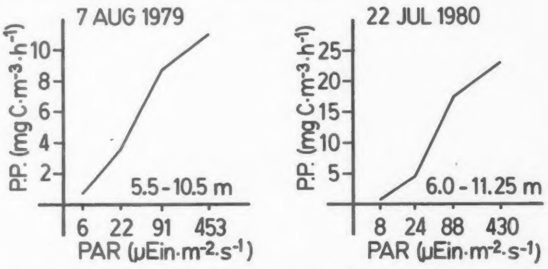


FIG. 7. Typical profiles of (a) relative PAR vs depth for L223, and (b) carbon uptake vs. PAR (measured in an incubator) for metalimnetic algal populations in L223 during midsummer. With surface irradiances ranging from 0 to approximately 2200 $\mu Ein \cdot m^{-2} \cdot s^{-1}$, irradiances below the mixed layer (>6.0 m) were almost always below saturation for carbon uptake (<250 $\mu Ein \cdot m^{-2} \cdot s^{-1}$).

Henrikson et al. (1980) showed that removal of all fish from a small lake can lead to apparent oligotrophication, including reduced limnetic primary production and increased transparency, via nutrient feedback mechanisms which ultimately affect the enire ecosystem. Many acidified lakes have lost their fish populations, but large numbers of fish still were present in L223 after three years near pH 5.1 (Mills et al. 1987). Thus, the specific mechanisms described by Henrikson et al. (1980) did not apply in this experiment. However, the disappearance, or even the reduction in numbers, of certain species higher in the food chain (Schindler et al. 1985) may have triggered "cascading trophic interactions" (Carpenter et al. 1985) which resulted in decreased grazing pressures and increased phytoplankton biomass and production.

Lake 223 differed from most acidified lakes in that the acid was added directly to the surface waters and not to the terrestrial watershed via precipitation. There was no opportunity for heavy metals to be leached by the acid from the terrestrial portion of the drainage basin or to be added directly to the lake along with the acid, as might occur with acidic precipitation. We expected direct acid responses in L223 rather than metal responses or synergistic acid-metal responses, although significant increases in the water-column concentrations of dissolved (<0.22 μm) aluminum, iron, zinc, and particularly manganese did occur (Schindler and Turner 1982; Schindler et al. 1987).

The Lake 223 experiment also differed from many studies in that the mean pH never dropped below 5.0. Nonetheless, at pH values well above 5.0, many detrimental effects of acidification on higher organisms and communities were observed (Schindler et al. 1985). The dramatic increase in maximum transparency, with a marked tendency towards greater fluctuations in transparency over time, might seem to support the oligotrophication hypothesis. However, the Lake 223 experiment has failed to demonstrate a reduction of phytoplankton production. This clearly contradicts the oligotrophication hypothesis and supports the findings reported by Dillon et al. (1979).

Although the acidification of L223 brought about changes in algal species (Findlay and Saesura 1980; Findlay 1984), the community continued to photosynthesize at rates equal to, or in excess of, those measured before acidification. These replacement species were apparently adapted to utilize the increased irradiances and to prosper under the more acidic conditions. Thus, while acidification might well have been detrimental to certain individual phytoplankton species, the net effect, in terms of the overall phytoplankton community, was an enhancement of productivity, at least during the early stages of acidification. This enhanced production was caused, at least in part, by decreased light attenuation in reponse to acidification. There is no evidence that gradual acidification to pH 5.0 by direct addition of sulphuric acid to the epilimnion led to oligotrophication in Lake 223.

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Vertical Distribution of Zooplankton in an Experimentally Acidified Lake Containing a Metalimnetic Phytoplankton Peak

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Kettle, W. D., M. F. Moffett, and F. deNoyelles, Jr. 1987. Vertical distribution of zooplanktoin in an experimentally acidified lake containing a metalimnetic phytoplankton peak. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 91 – 95.

A large biomass of phytoplankton occurred as a narrow band in the metalimnion of a small Canadian Shield lake which had been undergoing experimental acidification. This community represented the maximum concentration of algal biomass in the lake and was composed of small phytoplankton species. The diurnal distribution of crustacean zooplankton was determined in relation to this potential food source. *Cyclopoid nauplii, Cyclops bicuspidatus thomasi*, and *Daphnia catawba* remained diurnally associated with the peak while other species were uniformly distributed throughout the water column. Those species which remained diurnally associated with the peak were exposed to the greatest food concentration in the lake and were also exposed to lower acid conditions than those in the upper waters. A deep-dwelling phytoplankton community may offer a refuge for some zooplankton species in acid lakes by providing a large food supply under less acidic conditions. The importance of sampling acid lakes through the entire water column is supported by these observations.

Une importante biomasse de phytoplancton s'est formée en une bande étroite dans la couche métalimnéenne d'un petit lac du Bouclier canadien qu'on avait acidifié expérimentalement. Cette communauté représentait la concentration maximale de la biomasse des algues dans le lac et elle se composait de petites espèces de phytoplancton. La répartition diurne du zooplancton crustacée a été déterminée en relation avec cette source alimentaire potentielle. On a observé que les *Cyclopoid nauplii, Cyclops bicuspidatus thomasi* et *Daphnia catawba* sont demeurés au point de concentration maximale pendant le jour alors que d'autres espèces se distribuaient uniformément dans la colonne d'eau. Les espèces qui demeuraient dans la zone de plus grande concentration pendant le jour étaient exposées d'une part à une plus importante concentration d'aliments et d'autre part, à une plus faible concentration d'acide que celles qui se trouvaient dans la zone superficielle. Une communauté de phytoplancton vivant dans les grandes profondeurs dans les lacs acides, peut constituer un refuge pour certaines espèces de zooplancton en leur fournissant une source importante d'aliments dans un milieu moins acidifié. L'importance d'échantillonner les lacs acides par prélèvements dans toute la colonne d'eau est confirmée par ces observations.

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Acidification has produced many changes in lake ecosystems, including changes in plankton communities as reviewed by Haines (1981). Our study examines the vertical distribution of zooplankton in an experimentally acidified lake containing a deep chlorophyll maximum and considers the importance of sampling the entire water column at discrete depths to adequately examine these subepilimnetic communities in acidified lakes.

There are no reports on the diurnal vertical distribution of zooplankton in acidified lakes. There are, however, many reports on the diurnal vertical distribution of zooplankton in non-acidified lakes. During stratification in non-acidified lakes, many species of grazing zooplankton reside in the deeper waters by day and migrate to the surface waters at night where there is observed (or assumed) to be more food available (e.g. Brooks 1964; Hutchinson 1967; Cruz-Pizarro 1978; Orcutt and Porter 1983). The advantage of returning to the deeper waters during the day has been variously ascribed to such factors as the avoidance of visually orienting predators (e.g. Zaret and Suffern 1976) and the metabolic advantage of returning to colder water after feeding to increase growth efficiency (e.g. McLaren 1963, 1974). In lakes undergoing acidification, pH may be higher below the epilimnion than within the epilimnion (Kelley et al. 1982), but the effects of this on the vertical distribution of plankton are not known.

It is not always the case that more food resources are available in the epilimnion. Clear, stratified lakes can frequently contain deep-dwelling (subepilimnetic) phytoplankton communities whose biomass can be ten or more times that of the epilimnetic waters (Fee 1976; Richerson et al. 1978; Moll and Stoermer 1982; Priscu and Goldman 1983; Pick et al. 1984). Reports suggest that lake acidification may sometimes result in the formation of subepilimnetic phytoplankton communities which are composed of species more susceptible to zooplankton grazing (Schindler 1980; Findlay and Saesura 1980; Findlay 1984). The extent to which the crustacean zooplankton of lakes containing subepilimnetic chlorophyll maxima associate with and graze upon these phytoplankton communities has received little consideration in non-acidified lakes (Schindler and Novén 1971; Richerson et al. 1978) and none in acidified lakes. There are reports, however, of zooplankton associations with subsurface chlorophyll maxima in marine environments (Anderson et al. 1972; Herman 1983). Because deep-dwelling plankton communities may be concentrated in narrow bands, their presence and importance, particularly in acidified lakes, may not be adequately considered by integrated samples through entire strata or by samples from just a few discrete depths.

Materials and Methods

Lake 223 is a small oligotrophic lake in the Precambrian Shield of northwestern Ontario. The lake has an area of 27.3 ha, a mean depth of 7.2 m, and a maximum depth of 14.2 m. Experimental acidification of L223 began in 1976 and has been described by Schindler et al. (1980), Malley et al. (1982), and Schindler et al. (1985). On 5 September 1981 three buoys were anchored (20 m apart) in the east basin of the lake to establish sampling stations. The maximum depth at these stations was 14 m. Diel samples were collected on 9, 10, 18, 19 September and 1, 2 October 1981 for determination of selected physical/chemical conditions and for analysis of phytoplankton and zooplankton communities. Daytime sampling commenced at 1300 h and nighttime sampling at 2200 h.

A transparent Schindler trap (Schindler 1969) with a 30 L capacity, 0.5 m length, and 40 μm mesh net was used to sample crustacean zooplankton. A vertical profile at 1 m intervals was developed at each of the three sampling stations. The Schindler trap was lowered until the middle of the trap was at the given metre depth to be sampled. Samples were preserved in 4% formalin and enumerated with a stereomicroscope using 2 − 4 subsamples/concentrated sample. On average 41.7% ($\bar{x}$ ± 3.5%) of the total number of individuals in each sample were counted. Samples from one station, chosen randomly from the three sampled each day and night, were counted for 9, 10 September (while the peak was present at 10 − 11 m) and for 1, 2 October (after disappearance of the peak due to entrainment into the expanding epilimnion). Diel samples from the 2 m (an epilimnetic sample) and 10 m (the metalimnetic peak) depths were counted for each of the three stations to determine possible variation among stations. A detailed description of the composition of L223 zooplankton has been provided by Chang et al. (1981) and Malley et al. (1982) but only from analysis of samples from daytime vertical net hauls.

Fluorescence profiles for relative estimates of the vertical distribution of phytoplankton biomass were measured during the day and night using an *in vivo* flow through fluorometer system (Fee 1976). The vertical distribution of phytoplankton species as strata (epi-, meta-, or hypolimnion) was available from integrated samples taken through each stratum (Shearer 1978) during the routine monitoring of L223 (Findlay and Saesura 1980; Findlay 1984). In addition, 50 mL subsamples of lake water were taken directly from each Schindler trap sample, and preserved in acid Lugol's solution for our identification and enumeration of phytoplankton species (deNoyelles et al. 1980) from discrete depths throughout the water column and from the metalimnetic phytoplankton peak.

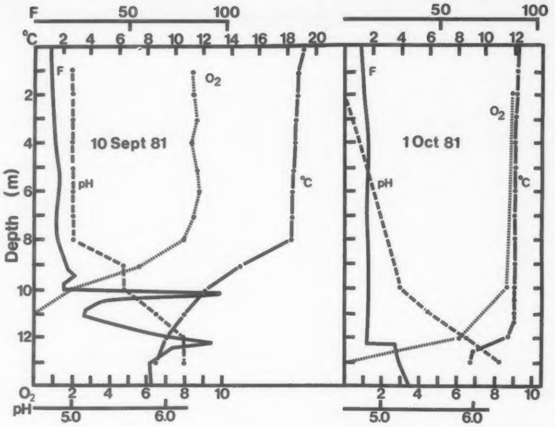

FIG. 1. Fluorescence (relative units), temperature (°C), oxygen (mg·L^{-1}), and pH profiles in Lake 223 before (10 Sept.) and after (1 Oct.) entrainment of the 10−11 m stratum into the epilimnion.

Because of the reports, described previously, suggesting that lake acidification may sometimes result in subepilimnetic phytoplankton communities composed of species more susceptible to zooplankton grazing, particular attention was paid to "edible" phytoplankton, defined as phytoplankton with all dimensions less than 35 μm and therefore edible by crustacean zooplankton (Burns 1968). Organisms with spines exceeding this dimension or with heavy silica shells (such as certain Diatomeae) were considered inedible. The concentration of edible phytoplankton within a stratum and at selected depths in the lake was calculated from the phytoplankton counts by volumetric conversion to wet weight biomass (Findlay and Saesura 1980).

Vertical profiles of temperature, light, pH, and oxygen were measured at 1 m depth intervals. Temperature was recorded with a YSI thermistor and light measurements were made with a quantum meter (Lambda Instr. Co., Model LI-185). Water for determination of pH (Radiometer model 29 pH meter) and dissolved oxygen (azide modification of Winkler Method) was collected with an 8 L Van Dorn bottle.

Results and Discussion

Fluorescence profiles revealed a distinct maximum 0.25 m wide at 10 − 11 m on 10 September (Fig. 1). On nine succeeding dates, through 28 September, fluorescence of this band was always at least six fold greater than values recorded for the epilimnion. The depth at which the band was located during this time period remained constant day and night. As in other lakes in this area (Fee 1976), the fluorescence peak was located in the metalimnion in the region of 0.5 − 1% surface illumination. Physical and chemical conditions in the peak (10 − 11 m depth) included: temperature 12.2 − 11.7°C, dissolved oxygen 2.2 − 0.7 mg·$^{-1}$, and pH 5.6 − 5.9. The peak remained at this depth until 27 September when a wind storm caused extension

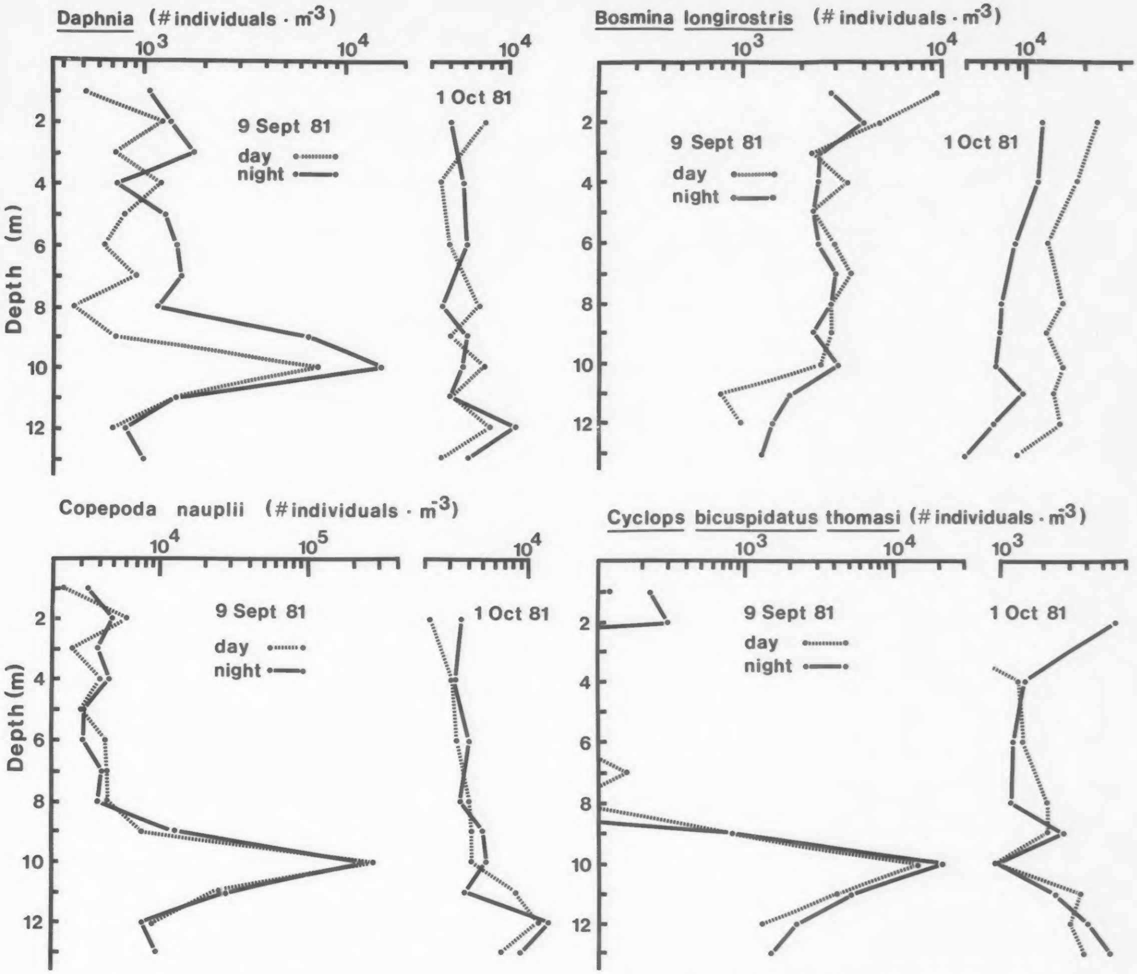

FIG. 2. Diurnal and vertical distribution of *Daphnia catawba*, copepoda nauplii, adults of *Cyclops bicuspidatus thomasi*, and *Bosmina longirostris* in Lake 223 before and after entrainment of the 10–11 m stratum.

of the epilimnion to a depth of 12 m which entrained the peak into the epilimnion. Until 17 September another peak was located at 12 m (Fig. 1) but was below the euphotic zone in the anoxic hypolimnion. This peak was composed of photosynthetic bacteria and detritus.

Metalimnetic chlorophyll maxima are common in many ELA lakes with the biomass generally composed of phytoplankton species, such as colonial Chrysophyceae, which are too large to be effectively eaten by crustacean zooplankton (Fee 1976; deNoyelles et al. 1980). The high fluorescence peak in the metalimnion of L223, however, was composed entirely of edible phytoplankton during September 1981. On 10 September an edible biomass (all dimensions <35μm) of 4300 mg·m^{-3} was recorded in the peak (representing 100% of the total biomass of the peak) compared to 650 mg·m^{-3} recorded at 2 m (representing 78% of the total biomass at that depth). From the routine strata samples, edible phytoplankton in the epilimnion was estimated at 1330 mg·m^{-3} on 31 August and 410 mg·m^{-3} on 14 September (D. L. Findlay, unpublished data). The edible biomass estimated for the metalimnion on 1 September (6.8 – 11 m) and 15 September (8 – 10.8 m) was 930 mg·m^{-3} and 1240 mg·m^{-3}, respectively. During September the peak was dominated by species of *Cryptomonas* (61% of total biomass of the peak) and *Chlamydomonas* (24% of total biomass of the peak). These species were localized in the peak since they represented less than 20% of the total biomass of phytoplankton in the integrated samples through the entire metalimnion on 1 and 15 September (D. L. Findlay, unpublished data). The magnitude of the conditions within the peak were obviously diluted with the integrated sampling of the entire stratum.

The diurnal vertical distribution of crustacean zooplankton in L223 was examined for the first time in September 1981. Herbivorous zooplankton species, including *Daphnia catawba* Coker and cyclopoid copepod nauplii, primarily *Cyclops bicuspidatus thomasi* S. A. Forbes and *Mesocyclops edax* (S. A. Forbes), were most abundant (day and night) in the 10 m samples (Fig. 2) and thus in the same stratum as the metalimnetic fluorescence peak. Adult *Cyclops biscuspidatus thomasi*, a crustacean zooplankton predator, was also most abundant at 10 m (day and night, Fig. 2). Statistical significance of diurnal distribution and of greater zooplankton abundance at 10 m was determined by performing Student's *t*-tests on 9, 10 September samples from the 10 and 2 m depths (six samples each, from three day and three night profiles). Densities of adult *Cyclops*, *Daphnia*, and nauplii were significantly greater ($P < 0.05$) at 10 m than at 2 m but not significantly different during day versus night at a given depth. Maximum densities (individuals$\cdot$L^{-1}) at 10 m for these species were 14.9 for *Daphnia*, 20.5 for *Cyclops*, and 275 for nauplii. The same distribution of these crustacean species was verified in the 18, 19 September samples.

For each of these species, their abundance at 10 m represented a disporportionately large percentage of their respective populations. For *Daphnia*, *Cyclops*, and nauplii, 33, 74, and 72% of their populations, respectively, occurred at the 10 m stratum (which as a 1 m stratum, 9.0 – 10 m, represented only 5% of the lake volume). We also noted that *Daphnia* at 10 m were significantly larger than individuals from all shallower sample depths ($P < 0.05$, GT2 multiple comparison tests where critical values for comparison limits were from a two-tailed studentized maximum modulus distribution). The mean body size of individuals at 10 m was 1.34 ± 0.06 mm (± SE, $n = 36$) compared to a range of 0.72 ± 0.06 mm ($n = 29$) to 1.02 ± 0.06 mm ($n = 36$) for all depths sampled above 10 m. Obviously failure to adequately sample this narrow stratum at 10 m in L223, or similar strata in other acidified lakes, could result in vastly different characterization of zooplankton populations.

Other crustacean zooplankton common in L223 on 9, 10 September but not concentrated at 10 m included *Bosmina longirostris* (O.F. Muller) (Fig. 2) and *Mesocyclops edax*; both were uniformly distributed throughout the water column both day and night. *Holopedium gibberum* Zaddach, *Diaptomus minutus* Lilljeborg, and cyclopoid copepodids were significantly ($P < 0.05$, Student's *t*-test) more abundant at 2 m than at 10 m. Abundance at 2 m (representing 13% of the lake volume as a 1 m stratum) was 2.6 individuals$\cdot$L^{-1} for *Holopedium*, 8.7 individuals$\cdot$L^{-1} for adult *Diaptomus*, and 3.6 individuals$\cdot$L^{-1} for the cyclopoid copepodids, which represented 26, 27, and 22% of their total populations, respectively.

On 1, 2 October, five days after the epilimnion deepened to 12 m, entraining the chlorophyll maximum, the dirunal distribution of species previously most abundant at 10 m was more uniform throughout the water column. *Daphnia* and *Cyclops* (Fig. 2) were not significantly more abundant in the deeper waters. The percent of the total *Daphnia* population at 2 and 10 m within the epilmnion and 12 m within the metalimnion was 14, 5, and 3%, respectively. For *Cyclops* these values were 8, 5, and 4%, respectively. Mean *Daphnia* lengths were not significantly different at different depths day or night. Only the Copepoda nauplii were significantly more abundant in the deeper waters (12 m compared to 2 m depths, $P < 0.05$, Student's *t*-test), but this association was less pronounced than on 9, 10 September (Fig. 2). All other zooplankton genera including *Bosmina* (Fig. 2), *Mesocyclops*, *Diaptomus*, *Holopedium*, and cyclopoid copepodids had densities evenly distributed throughout the water column.

The effects of lake acidification on diurnal vertical distribution of zooplankton has not been studied previously in L223 or in any other lakes to our knowledge. In the only other study of zooplankton vertical migration of ELA lakes, Schindler and Novén (1971) report for two lakes that most of the herbivorous crustaceans were located below the epilimnion at the strata of maximum phytoplankton biomass during the day and then migrated upward at night. In these two lakes *Daphnia catawba* vertically migrated and copepod nauplii did not migrate (cyclopoid adults were not monitored). Many species of *Daphnia* are known to migrate vertically (Hutchinson 1967), including *Daphnia catawba* (Tappa 1965), but vertical migration of cyclopoid copepods, including *Cyclops bicuspidatus thomasi* (eg. Hutchinson 1967; Patalas 1969), and copepod nauplii (eg. Hutchinson 1967; Zaret and Suffern 1976) is less common. Thus the distribution of *Daphnia* we observed in L223 seems particularly anomalous.

Malley et al. (1982) have described some changes in the L223 crustacean zooplankton in response to lake acidification. They suggested that the observed elimination of three species of crustacean zooplankton by 1980 was due to the direct toxic effects of hydrogen ion and that the general increase in abundance of herbivorous zooplankton might be related to greater food supply in the euphotic zone. The species of zooplankton recorded in L223 are similar to those recorded for other acidified lakes (Sprules 1975; Roff and Kwiatkowski 1977). *Daphnia catawba* was first reported in L223 in 1980 as *D. catawba* × *schoedleri* by Malley et al. (1982). This species has been found in other acidic lakes (Sprules 1975). The reason *Daphnia catawba* was able to become established after acidification is not known.

The association of the herbivorous zooplankton, *Daphnia* and the cyclopoid nauplii, with the phytoplankton peak described here may have been due, at least in part, to the exploitation of this food-rich resource. *Cyclops* may have associated with the peak due to the abundance of potential zooplankton prey. This species is a carnivore which feeds on rotifers, cladocerans and all stages of both Calanoida and Cyclopoida (McQueen 1969; Anderson 1970; Lane 1978). The experimental acidification of L223 may also have directly influenced the vertical distribution of zooplankton. For example, zooplankton could lessen their exposure to the most extreme pH at times of stratification by migrating below the epilimnion where pH declines are less due in part to greater natural buffering (Kelly et al. 1982). To our knowledge such migration has been reported only once (Grotervd 1972). After entrainment of the phytoplankton peak into the epilimnion in late September, those zooplankton species formerly associated with the peak were more evenly distributed throughout the water column. These species over the next several days did not return to the deeper waters in numbers comparable to those prior to entrainment even though a metalimnion containing oxygen and higher pH still existed now at 12 m instead of 10 m (Fig. 1). This may indicate that the vertical distribution of these zooplankters was more strongly influenced by the vertical distribution of the phytoplankton community than by the lower pH of the upper water.

Studies of phytoplankton and zooplankton response to lake acidification have not adequately considered the distribution of the plankton at discrete depths in the subepilimnetic portion of the water column. It is apparent from this study of an experimentally acidified lake that the subepilimnetic phytoplankton may provide a significant food resource for herbivorous zooplankton and that certain zooplankton species associate with this phytoplankton both day and night. The role of lake acidification in creating this condition and the effects this condition may have elsewhere in the lake can only be determined by incorporating an examination of subepilimnetic plankton communities into the research design of future studies.

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Reproductive Impairment of the Crayfish *Orconectes virilis* in Response to Acidification of Lake 223

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France, R.L. 1987. Reproductive impairment of the crayfish *Orconectes virilis* in response to acidification of Lake 223. Can. J. Fish. Aquat. Sci. 44 (Suppl. 1): 97–106.

Reproductive characteristics of crayfish (*Orconectes virilis*) were examined during 1979–81 in four small Canadian Shield basins in the Experimental Lakes Area, northwestern Ontario. One of these lakes, Lake 223 (L223), has been experimentally acidified since 1976. Egg resorption, fertilization, and rate of development were not seriously affected by lake acidification to pH 5.1. Incomplete hardening of the glair–cement compound forming the egg capsule membrane and stalk resulted in a loss of eggs from the pleopods, causing the L223 population to suffer decreased reproduction. On an egg production basis, the reproductive impairment (defined as the % decrease in number of viable eggs prior to hatching) in reference populations averaged 3.2 ± 1.8 (95% C.I.) compared to levels of 18.7, 36.2, and 29.4% during 1979–81 in L223 at pH 5.2–5.6. Direct mortality of eggs accounted for little of the reduced natality in the L223 population and did not occur in reference lakes. Loss of crayfish populations exposed to lake acidification will probably result from reproductive failure before lake water becomes acid enough to be directly toxic to mature crayfish.

Les caractéristiques reproductives de l'écrevisse (*Orconectes virilis*) ont été étudiées de 1979 à 1981 dans quatre petits bassins du bouclier canadien dans la région des lacs expérimentaux, au nord-ouest de l'Ontario. On acidifie expérimentalement un de ces lacs, le lac 223 (L223), depuis 1976. La résorption des œufs, la fertilisation et le rythme de croissance n'ont pas été affectés sérieusement par l'acidification du lac jusqu'à un pH de 5,1. Le durcissement incomplet du composé de ciment-glaire formant la membrane de l'oosphère et du pédoncule a entraîné une perte d'œufs venant des pléopodes, amenant une diminution de la reproduction dans la population du L223. Sur le plan de la production d'œufs, l'altération de la reproduction (définie comme la diminution en % du nombre d'œufs viables avant l'éclosion) dans les populations de référence a été en moyenne de 3,2 ± 1,8 (95% I.C.) par comparaison aux niveaux de 18,7, 36,2 et 29,4% de 1979 à 1981 atteints dans le L223 à un pH de 5,2 à 5,6. La mortalité directe d'œufs n'expliquait qu'une faible partie de la natalité réduite dans la population du L223 et ne s'est pas produite dans les lacs de référence. Il y aura probablement perte de populations d'écrevisses exposées à l'acidification du lac à la suite du déclin de la reproduction avant que l'eau du lac ne devienne suffisamment acide pour être toxique directement aux écrevisses adultes.

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Crustaceans are among the most sensitive organisms to lake acidification (Hendrey et al. 1976; Leivestad et al. 1976). Previous studies have been largely survey-oriented and have shown that distribution of crustaceans is curtailed below pH 6.0 (Okland and Okland 1985; Sutcliffe and Carrie 1973), or that communities experience reductions in diversity with increasing water acidity (Sprules 1975; Roff and Kwiatkowski 1977; Fryer 1980). Inherent difficulties exist, however, in establishing causal relationships between pH and survey data. Few North American investigations have monitored the long-term response of crustaceans to progressive acidification in a single lake (Yan and Strus 1980; Malley et al. 1981; Nero and Schindler 1983). Consequently, there are few data on the sublethal effects of lake acidification upon crustaceans, either benthic or planktonic.

The acidification of softwater lakes and rivers poses a serious threat to the long-term survival of the crayfish *Astacus astacus*, often considered to be the most economically important species in many Scandinavian waters (Svardson 1949). There are numerous references to *A. astacus* populations that have suffered from increasing acidity over recent years (Svardson *in* France 1981; Hultberg 1976; Appelberg 1980). Abrahamsson (1972) and Furst (1977a) have also documented several unsuccessful restocking attemps in lakes of low pH. In one case (Abrahamsson 1972), cessation of liming led to a failure of reproduction and extinction of crayfish when lake acidity increased to about pH 5.6. Despite the generally accepted belief that crayfish disappear from lakes as the pH drops below 6.0 (Larsen; Munthe-Kaas *in* Abrahamsson 1972; Almer et al. 1978), and that fishing yields have also decreased dramatically in such lakes (Svardson *in* France 1981), the mechanisms of population decline are not known. Recently, as a result of laboratory experiments, Appelberg (1981, 1985) suggested that failure in reproductive success could be one of the most important factors contributing to the low abundance of *A. astacus* in water below pH 6.0.

Contrary to the species *Cambarus robustus* (Collins et al. 1981; Berrill et al. 1985), laboratory work has indicated that several other North American Orconectid species, particularly

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Orconectes virilis (Hagen), are relatively sensitive to low pH in terms of iono-regulation, mortality, and behavioral modifications (Malley 1980; Morgan and McMahon 1982; France 1984; Malley and Chang 1985; Berrill et al. 1985; France 1985a, 1985b). The purpose of this study was to provide detailed information on the effects of lowered pH on fecundity of *O. virilis*, which together with data on prevalence of parasitism (France and Graham 1985) and inhibited calcification (France, unpublished data), would aid in interpretation of the effects of a whole-lake acidification experiment upon this species (Davies, unpublished data; Schindler et al. 1985).

Study Area

The Experimental Lakes Area (ELA) is situated at 93°30′−94°00′W and 49°30′−49°45′N, 300 km east of Winnipeg, Manitoba. ELA is a headwater region containing small glacially formed basins in the Precambrian Shield. Lakes are oligotrophic, ice-covered during November through May, and have dimictic thermal regimes. *Orconectes virilis* is the only crayfish species present in these lakes. Four ELA lakes were selected for detailed study of crayfish life history from 1979 to 1981: Lake 223 (L223), L224, L239, and L240. Morphoedaphic characteristics of these lakes of particular significance to crayfish are provided in France (1985c). Lake 223 has been experimentally acidified with sulfuric acid since 1976 to simulate effects of acid precipitation (Schindler et al. 1980; Schindler 1980; Schindler and Turner 1982; Schindler et al. 1985). The mean ice-free epilimnetic pH of L223 was decreased from about 6.8 in 1974−1976, to 5.6 in 1979, 5.4 in 1980, and 5.1 in 1981.

Methods

During spring and early summer (May 15−June 19) from 1979 to 1981, ovigerous *O. virilis* were collected from the four study lakes. Crayfish were gathered at night with a suction gun over a depth range of 0−6 m using SCUBA. Descriptions of collection sites and sampling methods are presented in France (1985c). Rostrum carapace length, weight, presence of eggs, cement gland development, and exoskeletal rigidity were recorded for each female.

The distribution of eggs among pleopods and overall general condition of the female and attached eggs were closely examined under a dissecting microscope. Counts of eggs were made after gently scraping and removing the eggs from pleopods with a blunt probe. The condition of pleopod egg development during 1980 was categorized into seven levels reflecting easily observed alterations in external color and morphological characteristics:

Level 1: newly-laid eggs characterized by a uniform black colour.
Level 2: eggs uniform black-grey.
Level 3: eggs grey with large light-brown spots developing.
Level 4: muddy-grey appearance with small polar concentration of purple-lavender colour.
Level 5: polar developmental disc becomes light-pink in colour, thoracic appendages becoming evident.
Level 6: "eyed-stage" with obvious orange carapace and black eye-spots forming just prior to hatching.
Level 7: eggs in process of hatching and a few attached S−I young.

Particular attention was directed to the occurrence and enumeration of abnormal conditions such as inviable eggs and/or empty egg capsules and stalks. Occasionally pleopods were removed and number of empty capsules and stalks enumerated. After removal of attached eggs, females were weighed, and estimates of mean relative fecundity (number of eggs per gram mass of female) were calculated. During 1979, a comparison was made of egg weight and diameter among the lakes.

Some females were sacrificed and ovaries removed and examined to investigate the occurrence and extent of egg resorption. During the spring of 1980 this was categorized into five levels reflecting general appearance of the ovary:

Level 1: ovary is a small, homogeneous, white-opaque mass of condensed particles, indicative of no resorption.
Level 2: ovary is voluminous with translucent white particles more distinct and spherical than Level 1, also indicative of no resorption with the white flecs representing formation of minute new eggs.
Level 3: progressively larger bi-lobed ovary with old eggs in late states of resorption and reduced to isolated irregular yellow masses scattered amongst the white newly forming oocytes; transition level.
Level 4: large ovary filled with oocytes of various sizes and colours representing different stages of resorption of yolk substance, characterized by distinctive presence of large orange or red semi-spherical oocytes of a similar size to successfully extruded eggs.
Level 5: occurrence of large black oocytes rich in vitellus and identical in size and shape to successfully laid eggs, indicative of the earliest stages of degeneration prior to resorption of released yolk substances; rest of ovary similar to Level 4.

In autumn of 1979 and 1980 (September 25−October 28), adult females were caught, ovaries removed, and counts made of all visibly developing eggs. The general condition of the female (i.e. occurrence of sperm plugs, cement gland development, and carapace rigidity) was also noted. Mature L223 and L224 females with obvious cement glands and sperm plugs were held in recirculated L239 water (pH 6.6−7.0) over winter until oviposition occurred in April 1981.

Results

Over 90% of the eggs on ovigerous *O. virilis* were fastened to the pleopods (France 1983; Fig. 1A). Eggs of L223 females could always be removed more easily than those from females from reference lakes.

Less secure attachment of pleopod eggs in L223 females resulted in a higher proportion of females with reduced egg numbers than in reference lakes (Fig. 2). In all cases there was a significant relationship ($P < 0.05$) between female carapace length and number of attached eggs (individuals with reduced number of eggs for a particular length are designated as open circles and not included in regression equations). Calculations of relative fecundity (number of eggs per g mass of female) of

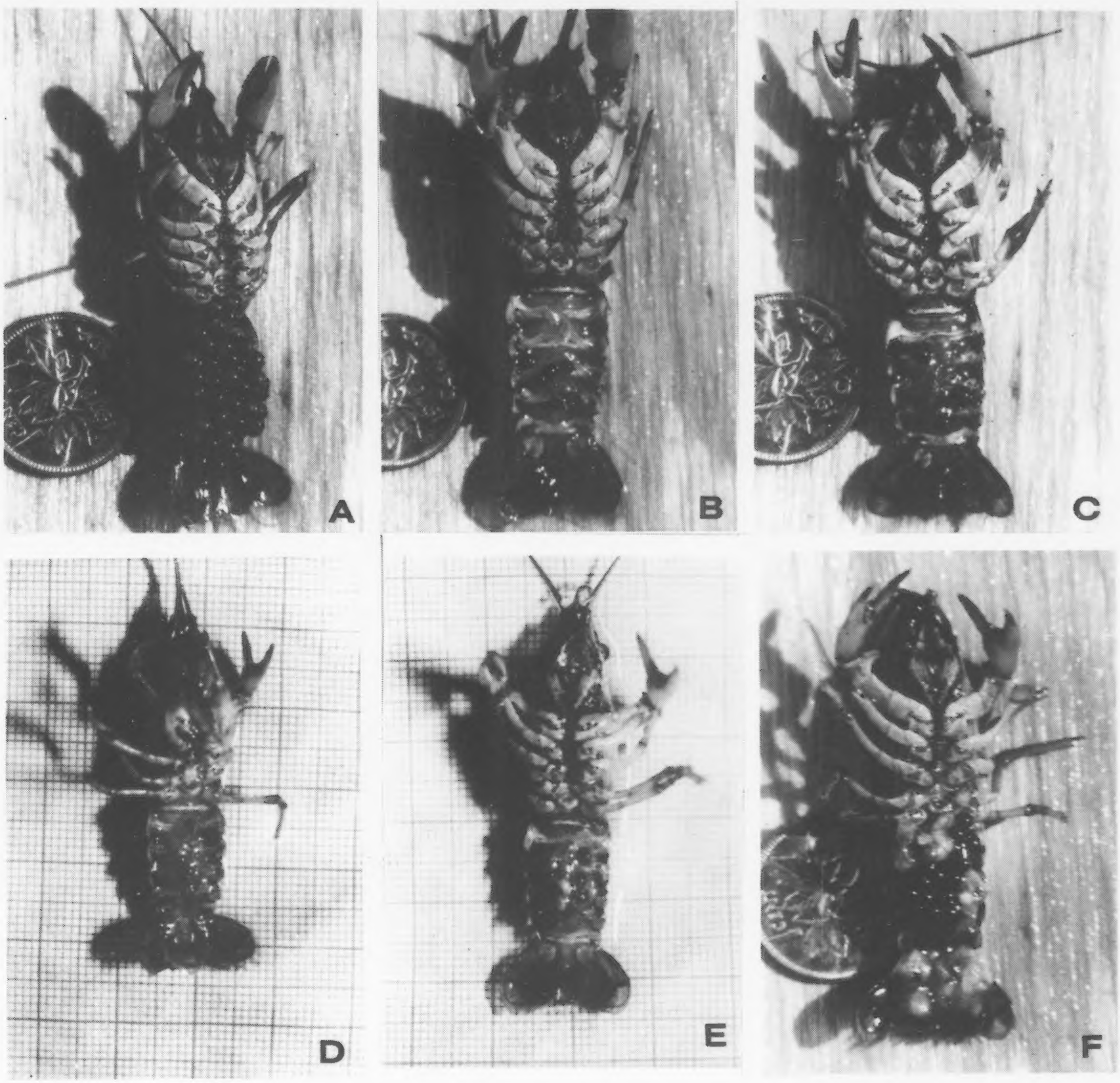
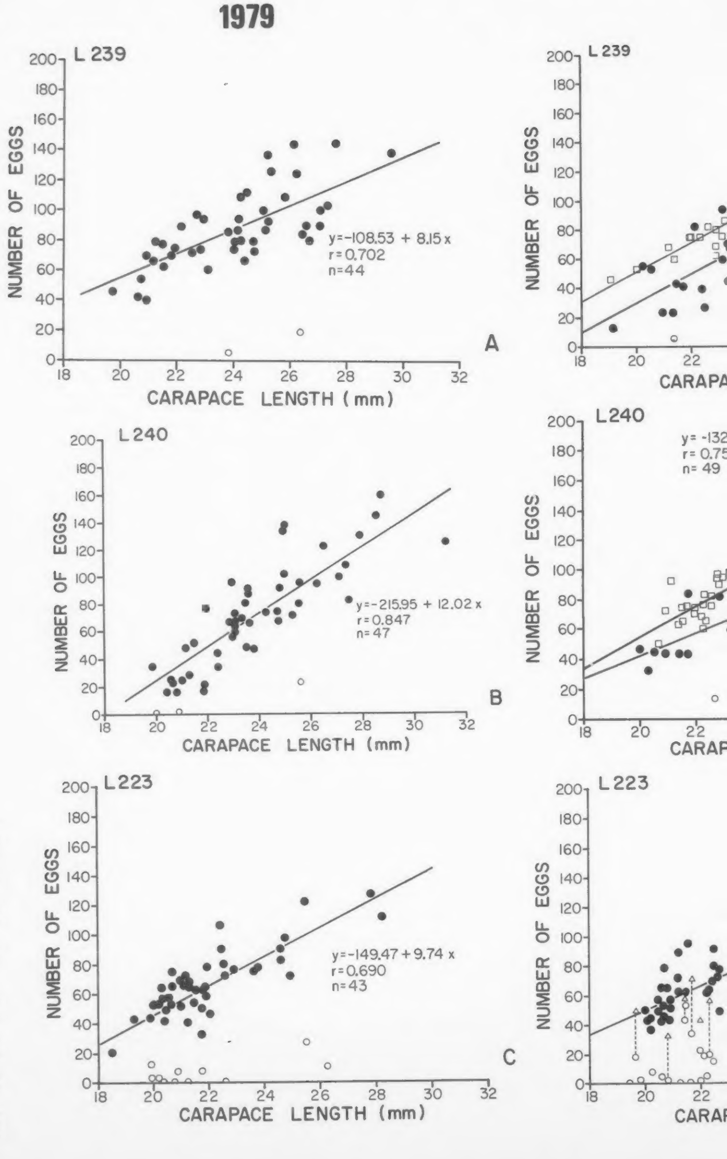


FIG. 1. Distribution of eggs over the abdomen of *O. virilis* from L223 with (A) a full complement; (B) the majority of the brood lost; (C), (D), (E) pleopods bound together with unhardened glair–cement; and (F) partial egg mortality. Colour photographs of C and F in Anon. 1981, 1984.

crayfish displaying no symptoms of reproductive stress (described below) showed no significant ($P > 0.05$) differences among lakes (Table 1). Alternatively, if all the ovigerous crayfish, including those with reproductive impairment, were included in the analysis, the estimate of relative fecundity for the L223 sample was significantly ($P < 0.05$) lower than that for 5 of 6 comparisons with samples from the reference lakes. The number of ovarian eggs produced by mature *O. virilis* was linearly correlated with female length and always greater than the number of eggs successfully extruded and attached to the pleopods the subsequent spring (Fig. 2D, E, G). The disparity or percentage drop in count between mean ovarian and pleopod eggs over a similar and restricted animal size range for each sample was two-fold greater in L223 than in reference lakes (Table 2)

Quantitative and qualitative comparisons of egg attachment indicated that L223 crayfish were distinct relative to those from the three reference lakes. The percentage of L223 females with a partial complement of eggs (open circles relative to closed circles in Fig. 2, recognized as described below) was 19.0% in 1979, 42.4% in 1980, and 47.7% in 1981 compared to levels of approximately 5% over the 3 years in reference lakes. The manner in which eggs were scattered over abdominal pleopods indicated that these few eggs were remnants of a much larger brood, the majority of which had been lost (Fig 1B, cf. France 1983). Often pleopods of these individuals were intertwined


1979
L 239
NUMBER OF EGGS
CARAPACE LENGTH (mm)
y=-108.53 + 8.15 x
r=0.702
n=44
A
L 240
NUMBER OF EGGS
CARAPACE LENGTH (mm)
y=-215.95 + 12.02 x
r=0.847
n=47
B
L 223
NUMBER OF EGGS
CARAPACE LENGTH (mm)
y=-149.47 + 9.74 x
r=0.690
n=43
C
L 239
NUMBER OF EGGS
CARAPA
L 240
y= -132
r= 0.75
n= 49
NUMBER OF EGGS
CARAP
L 223
NUMBER OF EGGS
CARAP

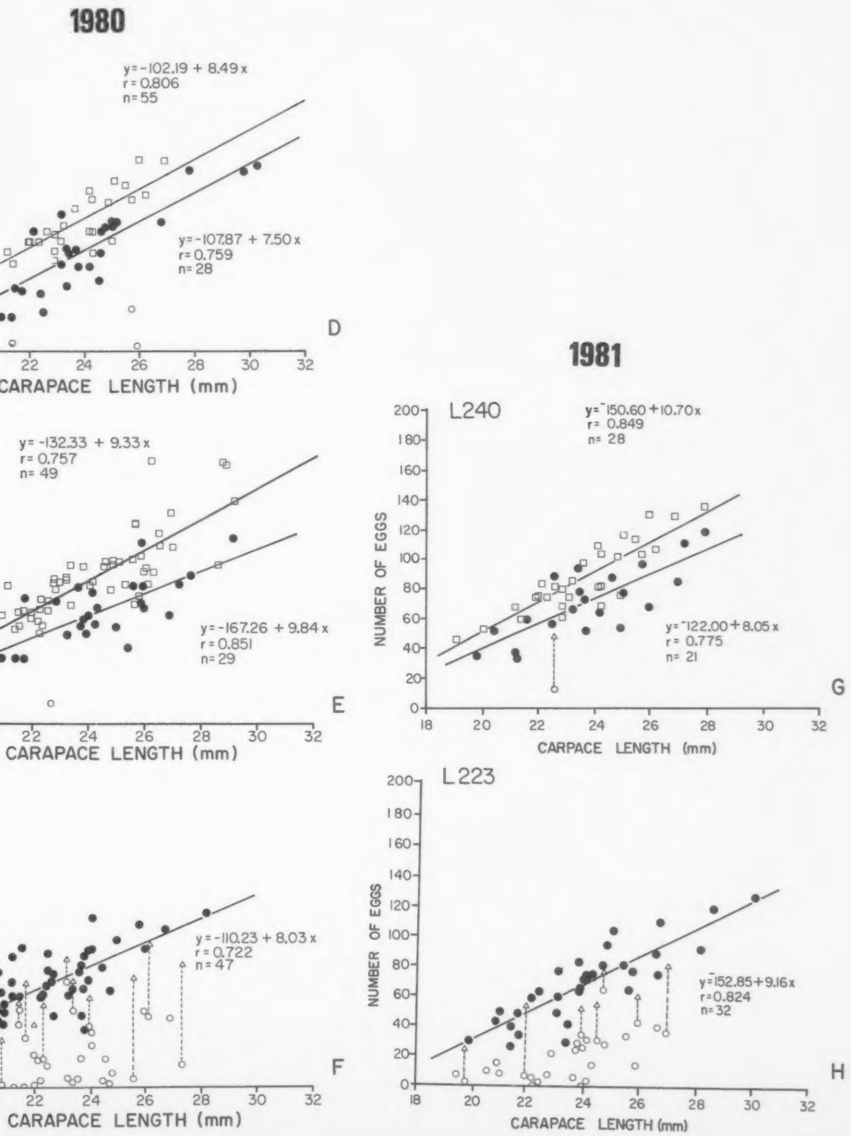
1980
y=-102.19 + 8.49 x
r= 0.806
n= 55
y=-107.87 + 7.50 x
r= 0.759
n= 28
D
22
24
26
28
30
32
CARAPACE LENGTH (mm)
y= -132.33 + 9.33 x
r= 0.757
n= 49
y= -167.26 + 9.84 x
r= 0.851
n= 29
E
22
24
26
28
30
32
CARAPACE LENGTH (mm)
y= -110.23 + 8.03 x
r= 0.722
n= 47
F
22
24
26
28
30
32
CARAPACE LENGTH (mm)
1981
L240
y= -150.60 + 10.70 x
r= 0.849
n= 28
y= -122.00 + 8.05 x
r= 0.775
n= 21
G
200
180
160
140
120
100
80
60
40
20
0
NUMBER OF EGGS
18
20
22
24
26
28
30
32
CARPACE LENGTH (mm)
L 223
y= -152.85 + 9.16 x
r= 0.824
n= 32
H
200
180
160
140
120
100
80
60
40
20
0
NUMBER OF EGGS
18
20
22
24
26
28
30
32
CARAPACE LENGTH (mm)

TABLE 1. Comparison of relative fecundity (no. eggs·g^{-1} mass of female) among study lakes. Data presented as mean ± SE (n) for each fecundity estimate; * = significantly ($p < 0.05$) different from L223 value. Successfully reproducing = only closed circles present in Fig. 2; all ovigerous = individuals with evident glair–cement or egg loss included, i.e. open circles from Fig. 2.

Year	Condition	L239	L240	L223	L224
1979	successfully reproducing	22.2 ± 0.7 (44)	19.7 ± 0.8 (47)	19.5 ± 0.7 (43)	—
	all ovigerous	21.1 ± 1.0 (46)*	18.6 ± 0.7 (50)	16.4 ± 1.0 (54)	—
1980	successfully reproducing	20.8 ± 1.2 (28)	19.8 ± 1.1 (29)	20.9 ± 0.8 (47)	20.4 ± 1.8 (13)
	all ovigerous	20.3 ± 1.2 (31)*	17.9 ± 1.1 (30)*	14.7 ± 0.9 (82)	20.4 ± 1.8 (13)*
1981	successfully reproducing	—	20.1 ± 1.5 (21)	19.8 ± 1.1 (32)	—
	all ovigerous	—	19.9 ± 1.5 (22)*	14.3 ± 1.2 (60)	—

TABLE 2. Disparity between potential and realized reproductive capacity of *O. virilis* in ELA lakes. Data for each egg count presented as mean ± SE (n).

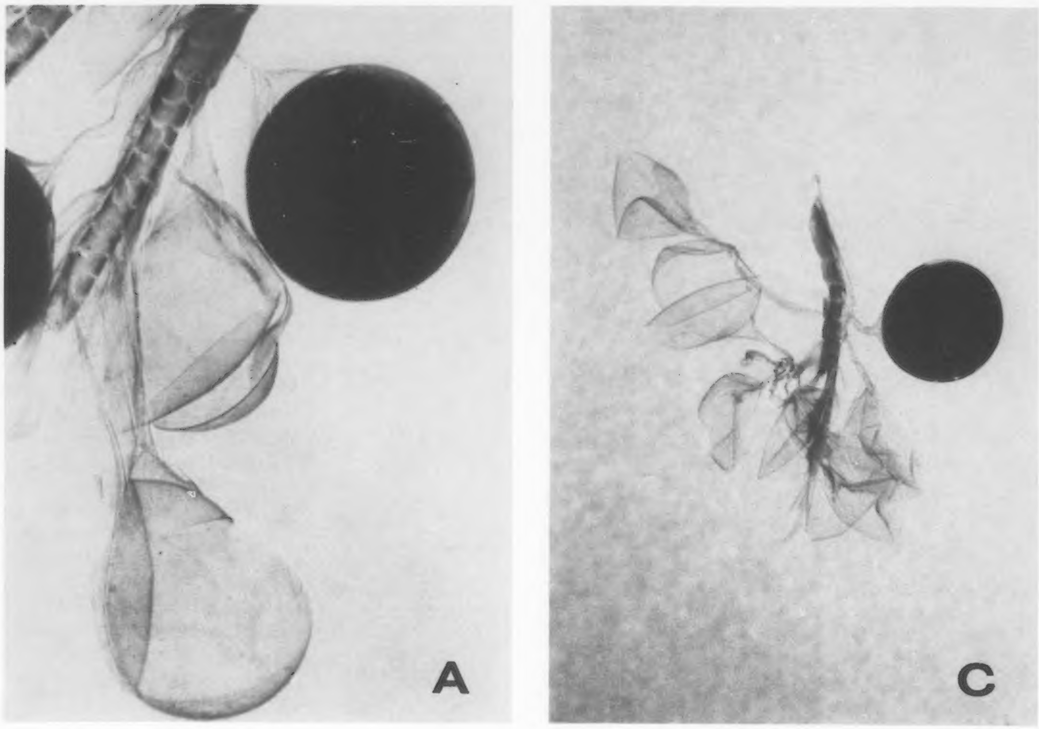
Year	Lake	Ovarian egg count	Pleopod egg count	Disparity (% drop in egg count)
1979–80	L239	98.1 ± 2.7 (55)	77.2 ± 4.6 (31)	21.3
	L240	88.3 ± 3.1 (49)	67.1 ± 6.4 (30)	24.0
	L223	88.7 ± 2.8 (43)	45.8 ± 5.8 (82)	48.4
	L224	77.7 ± 2.9 (31)	65.5 ± 6.3 (13)	15.7
1980–81	L240	86.2 ± 4.1 (28)	71.0 ± 5.1 (22)	17.6
	L223	91.8 ± 3.1 (44)	45.4 ± 6.4 (60)	50.6

and bound together into a single gelatinous mass by an amber-coloured substance (Fig. 1C, D, E). The glue-like nature of this viscous compound actually fixed pleopods together and occasionally completely enveloped the few remaining eggs. Closer examination under a dissecting microscope revealed this "glair-compound" (Andrews 1906) to be composed of numerous egg-stalks and empty capsules that had previously ruptured at the distal end and lost their eggs (Fig. 3A, B). The clear, hardened egg-stalk ligaments of female crayfish from reference lakes with normal attachment and number of eggs stretched markedly when pulled, and displayed great resilience when released. The amber-coloured and soft, amorphous L223 egg-stalk material, in contrast, pulled apart effortlessly, producing large gaps; it was not resilient. However, structural characteristics of glair–cement from L223 and L240 crayfish were not different when crayfish were incubated overwinter at neutral pH. Eggs of these crayfish were healthy and firmly attached to the pleopods with no indication of loss, mortality or resorption.

Empty egg cases were distributed evenly over all pleopod sets in L223 females (France 1983). Individual pleopods were uniformly covered by egg capsules along their entire length and on both endo- and exopodite branches indicating that egg loss was not restricted to the terminus (Fig. 3C). Summation of remaining eggs and empty capsules produced numbers close to those expected on the basis of the female's carapace length (Fig. 2F, H; and L240 Fig. 2G). This loss of eggs was not correlated with size of females and varied in its relative severity among individual crayfish. Where it occurred, egg loss was generally high; most females had lost over 50% of the potential brood (France 1983).

Egg mortality varied among crayfish as well as among pleopods on individual crayfish. The percentage of L223 females with dead eggs was 6.9% in 1979, 16.9% in 1980, and 13.8% in 1981; no females collected from reference lakes carried dead eggs. Such eggs were easily distinguished from healthy fertilized eggs by being bright orange or yellow and, depending when mortality occurred, they were often much smaller relative to successfully developing eggs. Usually non-viable eggs were scattered throughout the pleopod egg mass (France 1983). Occasionally, however, dead eggs formed distinct groupings about individual pleopod clusters, and were bound together into large discoloured masses surrounded by an amorphous, granular, opaque-white matrix which was proba-

FIG. 2. Relationships of carapace length to number of eggs in ELA crayfish during 1979, 1980, and 1981. Open squares denote ovarian egg counts during the autumn of the previous year and closed circles denote pleopod eggs in the spring of the year indicated. Open circles represent females with a reduced complement of pleopod eggs and were not included in the regression equations. Dashed lines connecting triangles with circles represent the difference between counts of successfully attached eggs and empty egg capsules for single individuals. Regressions of ovarian eggs to carapace length for L223 crayfish during 1980 and 1981 are not shown: $Y = -80.53 + 7.50x$, $r = 0.710$, $n = 43$; $Y = -105.13 + 8.50x$, $r = 0.849$, $n = 44$.



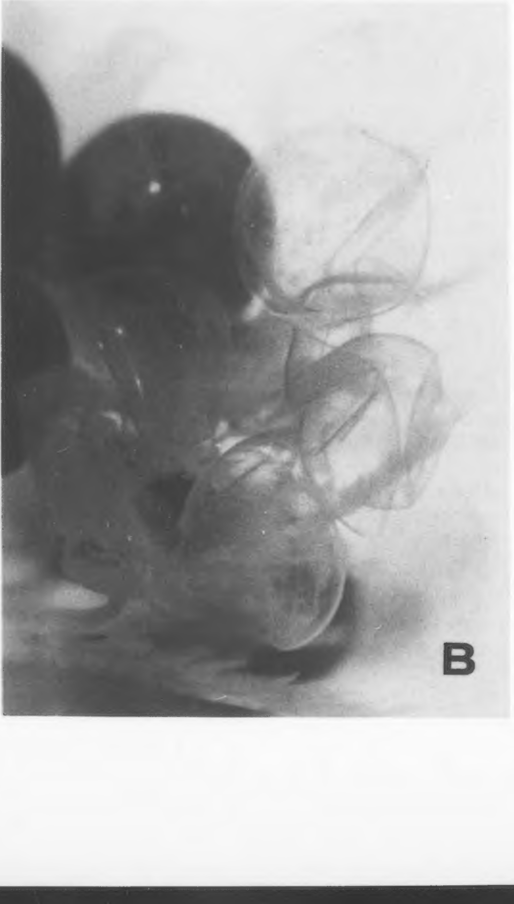


FIG. 3. Structure and distribution of empty egg capsules over pleopods from L223 crayfish.

bly a fungus (Fig. 1E). No qualitative difference in attachment of live and dead eggs to the same female was detected.

To circumvent the wide variability in egg loss or mortality among individual crayfish and obtain a more precise assessment of reproductive impairment, egg production was expressed on a population basis (Table 3). Reproductive impairment (defined as the % decrease in the number of viable eggs prior to hatching) in the three reference populations averaged 3.2 ± 1.8 (±95 C.I.) while values of 18.7, 36.2, and 29.4 were recorded during 1979–81 in L223. The majority of this impairment was contributed by egg loss as opposed to egg mortality.

Quality characteristics of successfully carried eggs were similar among study lakes. In 1979, egg size and weight in L223 were not significantly different from those in the three reference lakes (Table 4). Rate of egg development in crayfish from L223 during spring 1980 (pH = 5.4) was not different from that in a reference population (Fig. 4).

Fertilization of both ovigerous females and extruded eggs appeared unaffected by lake acidification. Mating pairs of *O. virilis* in L223 were observed in fall 1980 at a lake pH of 5.4, and during spring and fall 1981 at a lake pH of 5.1. Formation of sperm plugs throughout the fall and winter and their subsequent absence in ovigerous females collected the following spring implied successful copulation. Furthermore, the percentage of mature fertilized females in the L223 population was not unusual (France 1985c). The sum of viable eggs plus empty capsules for L223 female crayfish was only slightly smaller than that expected for crayfish of the same size in other

TABLE 3. Reproductive impairment of *O. virilis* at ELA calculated on a total egg production basis per population.

Reproductive characteristic	Control lakes 1979–81 $n=6$	Lake 223 1979	Lake 223 1980	Lake 223 1981
Total number of eggs examined	12132	2685	3963	2695
Extimated number of empty capsules	435	605	1760	993
% eggs lost	3.2 ± 1.8	18.3	30.8	26.9
Number of dead eggs	0	11	314	90
% eggs dead	0	0.4	7.9	3.3
Population % reproductive failure prior to hatching	3.2	18.7	36.2	29.4

lakes. This suggests that pH values as low as 5.1 probably do not severely reduce egg fertilization.

Ovarian egg resorption in L223 crayfish was elevated relative to reference lakes. In the reference lakes (L239 and L240 combined data) 10.0% of the sampled female population were resorbing ovarian eggs compared to 29.4% in L223 (Fig. 5). The number of these eggs being internally resorbed, however, was low (<5% of the total successfully extruded and attached for each crayfish). Egg resorption in L223 crayfish occurred independently of either pleopod egg loss or mortality.

TABLE 4. Crayfish egg quality parameters in ELA study lakes during 1980. Data presented as mean ± SE (n). There was no significant ($P > 0.05$) difference among lakes.

Lake	Egg diameter (mm)	Egg dry weight (mg)
L223	2.31 ± 0.08 (55)	1.55 ± 0.05 (39)
L224	2.24 ± 0.10 (58)	1.48 ± 0.09 (15)
L239	2.36 ± 0.04 (25)	1.49 ± 0.03 (42)
L240	2.20 ± 0.06 (73)	1.54 ± 0.04 (40)

Discussion

Reproductive "efficiency" of crayfish is generally low (Mason 1977). The number of ovarian eggs in *O. virilis* was always higher than the number of embryos successfully extruded and attached to pleopods the following spring. The increased disparity between these egg counts for L223 crayfish relative to those in reference lakes, resulted from a much higher prevalence of ovigerous females with reduced numbers of attached eggs in L223, the degree of reduction increasing as the lake was progressively acidified.

Discrepancies between ovarian and pleopod egg counts can be attributed to one or more of: (1) defective attachment of extruded eggs to pleopods, (2) mortality of attached eggs, (3) failure of fertilization, (4) incomplete extrusion of all eggs, or (5) other causes such as maternal cannibalism.

Egg Attachment

Oviposition in crayfish has been examined previously and little loss of eggs occurs normally during this period (Andrews 1906; Mason 1970a; Morrissy 1970). The most significant effect of lake acidification upon *O. virilis* reproduction was the failure of secure attachment of pleopod eggs in L223 females. This resulted in a reduced total fecundity in L223 crayfish compared to those from reference lakes. Interestingly, Appelberg (1985) in laboratory experiments also found significantly fewer attached eggs in the Swedish species *A. astacus* exposed to pH 5.0 compared to crayfish spawning at pH 6.0 and 7.0. Eggs lost from capsules will die because they require maternal pleopod activity for aeration and protection from fouling (Andrews 1906).

The egg-stalk begins as a flat, exceedingly delicate band of material, and with subsequent hardening becomes a narrow, twisted stalk of great tensile strength, forming a thick elastic outer case around the developing egg. It normally requires great effort to separate this stalk from the setae (Lereboullet 1860; Andrews 1906). Yonge (1946) describes in detail the importance of both firm consistency and marked elasticity of egg membranes to successful reproduction in lobsters. Lake 223 eggs could be easily removed from maternal pleopods because of the absence of these crucial properties.

The outer egg membrane and binding cement have properties indistinguishable from those of cuticle (Yonge 1937), and the processes involved with their formation are merely modifications of those present in the carapace following ecdysis (Lloyd and Yonge 1940; Cano *in* France 1981). Because decapod eggs and egg membranes are known to demonstrate decreased permeability at low pH (Lloyd and Yonge 1940; Appelberg 1985), the lack of structural resilience and subsequent adherence of eggs to the pleopod setae in L223 crayfish probably reflects an incomplete cuticular hardening of the glair–cement forming the egg capsule membrane and stalk. Similar processes involved with the synthesis and hardening of other structural layers in crayfish or lobsters are known to be extremely sensitive to depressed water pH (Malley 1980; Appelberg 1980; Morgan and McMahon 1982; Yonge 1937).

Four further lines of evidence corroborate the hypothesis of association of egg loss in L223 to low pH. These include: (1) The overwintering of L223 and L240 crayfish at neutral pH shows that unhardened glair-cement is not a normal characteristic of L223 females and must therefore be attributed to some factor(s) associated with the lake acidification experiment. Appelberg (1985), too, reported similar characteristics of the mucus secreted by cement glands of *A. astacus* ovipositing at low pH. (2) The symptoms of incomplete capsular hardening observed in L223 crayfish agrees with detailed visual descriptions from others of similar processes of reduced cuticular tanning or egg membrane formation (cf. France 1983). (3) Because the developmental progression of *O. virilis* embryos was unaltered by lake acidification to pH values between 5.1–5.4, the basic parameters of egg quality affecting survival, i.e. size and weight (Thorson 1950) have remained unchanged by L223 acidification. This implies that excessive uptake of water (Balasundaran and Panadian 1982) by L223 eggs cannot be the mechanism of increased recruitment failure

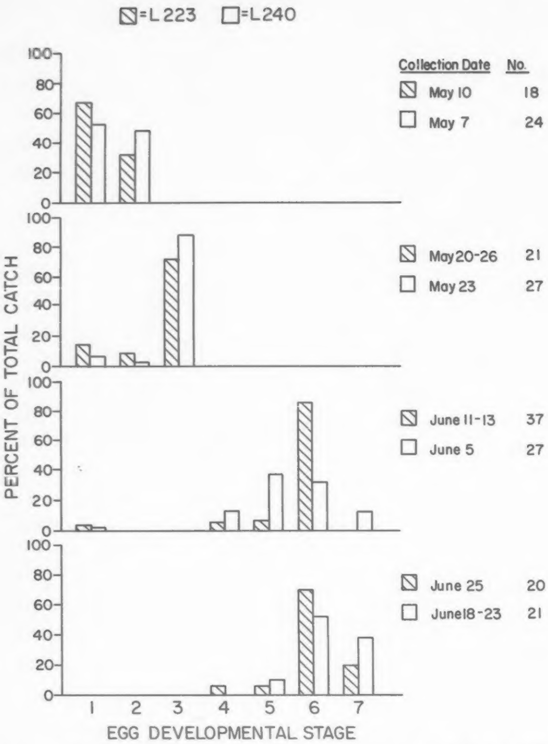


FIG. 4. Comparison of the rate of egg development through 7 stages (described in text) between L223 and L240 crayfish during spring 1980.

in that lake. (4) Due to actions of maternal grooming and feeding by recent hatchlings, the presence of ruptured egg capsules during brooding is an abnormal condition that occurs only when the young have been artifically removed (Andrews 1907; Mason 1970b, 1977).

Egg Mortality and Fertilization

Crayfish egg mortality is usually associated with fungal infection (Mason 1977; Vey 1977). The cause(s) and mechanism(s) of enhanced egg mortality in crayfish exposed to lake acidification are not well understood. Whether fungal egg infections are the direct cause of mortality (Fisher and Wickham 1976), or whether they occur after egg death (Woodland 1967), is not known. The possibility that mortality could be due to decreased maternal pleopod oxygenation of developing eggs, especially critical in acid environments (Appelberg 1981), was examined in the laboratory and dismissed (France 1985a). Regardless of the mode of toxicity, direct mortality accounted for only 2–15% of the reproductive impairment observed over 3 years in L223 at pH 5.1–5.6.

Likewise, the possibility that inihibition of fertilization, gametogenesis, and motility and viability of sperm could have significantly contributed to decreased *O. virilis* reproduction in L223 seems remote compared to the much more serious effects of egg loss. This may be in contrast to the situation with *A. astacus* (Appelberg 1985) which lacks an *annulus ventralis* so that the spermatophores remain in a relatively exposed condition compared to the situation in Orconectid crayfish (W. Momot, pers. comm.).

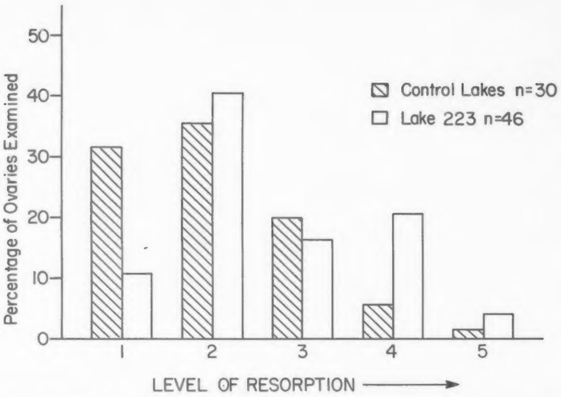


FIG. 5. The percentage of different levels of ovarian egg resorption in crayfish from L223 and reference lakes during spring 1980. For explanation of resorption index see text.

Several studies have indicated that infertile or moribund eggs are easily lost, leaving only the ruptured capsules and stalks attached to the pleopods (Woodland 1967; Fisher and Wickham 1976; Aiken and Waddy *in* Ennis 1980). However, loss of eggs from L223 crayfish occurred independently of egg viability and was not merely due to jettisoning of dead eggs, i.e. crayfish with a full complement of securely fastened eggs, and no empty capsules still carried dead eggs, whereas the remaining eggs of crayfish with many empty capsules were often all healthy. Small moribund eggs (implying early death) were successfully carried by females throughout development of the remaining healthy eggs, indicating that they are not preferentially lost.

Egg Resorption

Beamish et al. (1975) proposed that a failure of normal Ca metabolism caused mature fish in acid waters to resorb unlaid oocytes. Resorption of ovarian eggs and subsequent mobilization of yolk substances into the blood can occur in crayfish subjected to physiologically stressful conditions (Stephens 1952; Vey 1977). Atresia, however, was of little ecological importance in L223 as it only increased the total percentage of unlaid eggs from 0.4% (in reference situations) to 1.3% (in L223). Further evidence (France 1983) suggests that *O. virilis* in acid environments may actually attempt to form and develop their full complement of eggs with Ca obtained at the expense of exoskeleton hardness, via processes of internal carapace breakdown.

Possible Consequences

Experimental acidification of L223 has impaired reproduction substantially compared to other lakes in the ELA region. Absence of secure attachment and subsequent loss of pleopod eggs, probably due to incomplete hardening of the glair–cement, accounted for 85–98% of the impairment. The extreme sensitivity of *O. virilis* reproduction long before hatching occurs in June, poses a serious threat to the continued existence of these populations. In south-central Ontario *O.*

virilis oviposits in late April (Berrill 1978), a time coincident with spring melting of snow and pH depressions in receiving waters (Jeffries et al. 1979). Similarity of age frequencies between the L223 and reference populations in spring 1979 (France 1983), indicates that recruitment had not been reduced by acidification prior to that date. To ensure the long-term survival of *O. virilis* populations, a pH of no lower than 5.8 seems desirable; acidification to pH 5.1–5.6 detrimentally affected reproduction in L223. The L223 crayfish population was shown to decline to only a few percent of preacidification values by late fall 1981, and to possible extinction by fall 1983 at pH's between 5.0 and 5.1 (Davies *in* Schindler et al. 1985). In contrast, *C. robustus* is much more tolerant, and can maintain substantial abundance in softwater lakes at or below pH 5.0 (Collins et al. 1981; Berrill et al. 1985).

In this study, I measured only the decrease in viable eggs *prior* to hatching. Embryonic mortality and hatching failure increased below about pH 5.5 for *A. astacus, O. propinquus* and *O. rusticus* (Appelberg 1985; Furst 1977b; Berrill et al. 1985). Also, following hatching, stage I young should normally remain firmly connected to the maternal females by an elastic telson thread originating from the egg membrane that previously enclosed the embryo (Andrews 1907; Mason 1970b). Preliminary experiments with *O. virilis* hatching in low pH water, however, indicated a lack of secure attachment (D. Malley, FWI, unpublished data), which would prove lethal in nature (Andrews 1907). If these additional stresses are acting on *O. virilis* populations, the total recruitment of new individuals could, of course, be much lower.

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Calcium and Trace Metal Composition of Crayfish (*Orconectes virilis*) in Relation to Experimental Lake Acidification

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France R. L. 1987. Calcium and trace metal composition of crayfish (*Orconectes virilis*) in relation to experimental lake acidification. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 107–113.

Subjective estimates indicate that the carapaces of crayfish from experimentally acidified Lake 223 (pH 5.4–5.6) in the Experimental Lakes Area are becoming less rigid. Decreased carapace rigidity was inversely correlated with carapace dry weight and Ca^{++} content. *Orconectes virilis* from L223 have 25–35% less Ca^{++} in their exoskeletons (mean % dry wt ± SE = 13.90 ± 0.54) than do those from reference lakes (19.82 ± 0.33, 20.34 ± 0.63, and 22.18 ± 0.51). Lake 223 crayfish have accumulated higher tissue concentrations of both Mn (L223 value of 240 $\mu g \cdot g^{-1}$ dry wt compared to a mean ± SE for reference populations of 48 ± 11 $\mu g \cdot g^{-1}$ dry wt) and Hg (L223 value of 0.52 $\mu g \cdot g^{-1}$ dry wt compared to reference mean of 0.26 ± 0.05 $\mu g \cdot g^{-1}$ dry wt). Mn content of carapaces in crayfish from acidified L223 were also elevated threefold over background levels for the ELA region.

Des estimations subjectives révèlent que la carapace des écrevisses peuplant le lac 223, artificiellement acidifié (pH 5, 4–5, 6), de la Région des lacs expérimentaux (RLE) devient moins rigide. La baisse de rigidité présente une corrélation inverse avec le poids sec et la teneur en Ca^{++} de la carapace. L'exosquelette des *Orconectes virilis* du lac 223 contient de 25 à 35 % moins de Ca^{++} (% poids sec moyen ± É.-T. = 13,90 ± 0,54) que celui des écrevisses peuplant des lacs témoins (19,82 ± 0,33; 20,34 ± 0,63; 22,18 ± 0,51). Les écrevisses du lac 223 ont accumulé des concentrations somatiques plus élevées de Mn (240 $\mu g \cdot g^{-1}$ poids sec dans le lac 223 v. 48 ± 11 $\mu g \cdot g^{-1}$ poids sec (moyenne ± É.-T.) chez les populations témoins) et de Hg (0,52 $\mu g \cdot g^{-1}$ poids sec dans le lac 223 par rapport à une moyenne témoin de 0,26 ± 0,05 $\mu g \cdot g^{-1}$ poids sec). La teneur en Mn de la carapace des écrevisses du lac acidifié 223 est aussi trois fois plus élevée que la teneur ambiante dans la RLE.

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Disturbances in Ca^{++} metabolism leading to inhibited calcification or structural deformation of carbonate body parts have been observed in many animals exposed to acid water (Jewell 1922; Beamish et al. 1975; Hultberg 1976; Singer 1981; Nelson 1982; Fraser and Harvey 1982). For crustaceans, Borgstrom and Hendrey (1976) proposed that the effect of low pH may be to interfere with the uptake of Ca^{++} at molting. It has long been known in aquaculture practice that crayfish in acid waters tend to have thinner shells (e.g., LeCaze 1970). Appelberg (1980) found a significant decrease in dry weight and Ca content of *Astacus astacus* carapaces with decreasing pH. Similarly, *Orconectes virilis* held at pH 5.0 in ELA water for 10 d had slower rates of calcification and lower Ca contents than crayfish held at pH ≥6.0 (Malley 1980). It is not known if these results would be expected to be duplicated in non-laboratory environments.

The crayfish *O. virilis* is common in northwestern Ontario lakes that average 1.6 $mg \cdot L^{-1}$ Ca (Armstrong and Schindler 1971), and may be existing close to its lower environmental limit (Greenaway 1974; Capelli 1975). Therefore, relatively minor disruptions in Ca metabolism might be a critical factor for maintenance of crayfish populations. The close interrelationships between Ca and pH necessary for crustacean survival have been demonstrated previously in other systems (Sutcliffe and Carrick 1973; Okland 1980).

The geochemistry of trace metals in freshwaters is now widely known to be altered by acid deposition causing increases in their concentrations in acidified lakes (Dickson 1975; Schindler et al. 1980a). Despite these changes in water concentration, little is known about the effects of acidification on the metal concentrations of aquatic biota, and the consequences of these changes for populations in nature.

The metal Mn increases greatly in acidified lakes (Dickson 1975; Beamish and Van Loon 1977; Schindler and Turner 1982), and seems to be accumulated to a much higher degree in fish from such habitats compared to fish from circumneutral lakes (Lockhart and Lutz 1977; Fraser and Harvey 1982; Moreau et al. 1983). Field investigations suggest that uptake by fish of other trace metals such as Hg are accelerated at low lake pH (Scheider et al. 1979; Tomlinson et al. 1980). Although individuals of *O. virilis* are known to accumulate certain metals at a rate dependent on their external concentrations (Verner 1972; Anderson and Brower 1978), body metal concentrations in acidified waters have not been studied.

The purpose of this study was to document the calcium and

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TABLE 1. Mean ice-free epilimentic trace metal concentrations ($\mu g \cdot L^{-1}$) for ELA study lakes (values for L239 are for lake outflow). All data from D. W. Schindler (unpublished) except for L240 from Beamish et al. (1976).

Lake	Year	Mn	Al	Zn	Cu	Cd	Pb
L224	1976–77	3.8	11.0	2.4	1.2	<1	<1
L239	1976–77	5.4	—	3.9	1.1	<1	<1
L240	1975	—	—	<2	<2	<1	<1
L223	1980	127.0	27.0	5.6	1.8	<1	<1

trace metal concentrations in a population of *O. virilis* in Lake 223, an experimentally acidified lake in the Experimental Lakes Area (ELA) of northwestern Ontario (Schindler et al. 1985), and to compare these to similar data for crayfish from three reference lakes.

Description of Study Lakes

To simulate the effects of acid precipitation the pH of Lake 223 (L223) was decreased from natural values of 6.5–6.8 in 1976 to 5.4–5.6 by 1979 and 1980 at the time of this study. Details of lake acidification are given by Schindler et al. (1980b), Schindler and Turner (1982) and Schindler and others (1985). The whole-lake average concentration of Ca in L223 ($\simeq 2.2$ $mg \cdot L^{-1}$) remained unchanged by experimental acidification. Three other lakes (L239, L224, L240) are circumneutral and considered to be reference lakes for the region (cf. France 1985) with Ca concentrations between 1.7 and 2.4 $mg \cdot L^{-1}$ (Prokopowich 1979).

Trace metal concentrations in ELA waters are low, characteristic of an unpolluted region (Beamish et al. 1976; Table 1). However, as a result of the acidification of L223, several increases have been observed in volume-weighted average concentrations of metals. The most dramatic change has been a steady increase in Mn concentration from 12.5 $\mu g \cdot L^{-1}$ in 1976 to 127.0 $\mu g \cdot L^{-1}$ in 1980. Increases in Al from 15.4 to 27.0 $\mu g \cdot L^{-1}$, and Zn from 1.9 to 5.6 $\mu g \cdot L^{-1}$ are also attributed to lake acidification, while concentrations of Cu, Cd, Cr, and Pb remained near lower detection limits (D. W. Schindler, unpubl. data).

Methods

Carapace Rigidity and Calcium Content

Crayfish were sampled by SCUBA diving with the aid of a suction gun designed to prevent mechanical damage to specimens. Details of sampling methods are presented in France (1985). The rigidity of postmolt and intermolt crayfish was subjectively assessed by gently squeezing the carapace (I. J. Davies, Freshwater Institute, unpublished data). A six-level gradation progressing from very soft (VS), soft (S), intermediate soft-hard (S/H) and hard-soft (H/S), hard (H), to very hard (VH) was adopted. All determinations were performed by the author. Reassessment of individual crayfish indicated that the precision of the technique was over 95%. Comparative determinations on the same crayfish by another experienced researcher (I.J. Davies) using the same technique, generally agreed. Collections of between 193 and 294 crayfish for estimates of carapace ridigity were made from the four study lakes during autumn 1979 and 1980 following the last period of molting (France 1985). The proportion of the total catch that had lost or were regenerating one or both chelae was also calculated from the autumn 1980 collections.

During early July 1980, late postmolt and intermolt crayfish were collected for analysis of exoskeletal Ca^{++}. Because the concentration of exoskeletal Ca^{++} increases with crayfish size (McWhinnie et al. 1969; Greenaway 1974) only adults within the size range 23–27 mm carapace length were analyzed. Calcium content was determined using procedures similar to those described by Malley (1980). Portions of the carapace branchiostegite were removed with a paper punch, and both carapace and disc were dried for 24 h at 100 ± 5°C to obtain dry weights. Discs were then ashed at 500 ± 30°C for 24 h, dissolved in 0.25 mL of 12 N HCl, and brought to 25 mL with deionized water. Calcium concentrations of solutions were determined by atomic absorption spectrophotometry (Stainton et al. 1977). The ratio of carapace dry weight to length was calculated as an index of organic material (Huner et al. 1978).

Trace Metal Accumulation

Crayfish were collected from the study lakes 1 wk prior to ice formation in autumn 1980. Adult (age 2–3; 20–30 mm carapace length) crayfish of both sexes were frozen within 1 h of returning to the ELA field station. The tail-sections, including abdominal muscle and exoskeletal covering, were dissected from the remaining body with stainless steel instruments. This body section was chosen because it provides some integration of both internally accumulated and surface adsorbed metals that could be available to fish, as well as because previous work has suggested that the highest tissue concentrations in crayfish are found here (e.g. Hamilton 1972b). For each population, a homogeneous sample was prepared by pooling the abdomens of 20 to 30 individuals, then drying at 100°C for 24 h, and finally grinding these into a fine powder in a sample blender. Although only based on single measurements (2 replicates per analysis) for each population, the fact that there was both a close agreement in tissue concentrations with previous determinations on crayfish from L240, and a demonstrated correlation between increasing metal levels in both water and crayfish in L223 through acidification, reinforced confidence in any differences between populations in average tissue concentration. Determinations of tissue concentrations of Mn, Al, Zn, Cu, Cd, Pb, Hg, and Se via atomic absorption spectrometry were carried out by the Analytical Chemistry Unit at the Freshwater Institute, Winnipeg (cf. Nero and Schindler 1983). Manganese was also analysed by atomic absorption from carapace discs previously prepared for Ca measurements.

Results

Carapace Rigidity and Calcium Content

The median carapace rigidity of crayfish from Lake 223 was lower than those from reference lakes (Fig. 1). During autumn 1979, the majority of crayfish in reference populations were in the VH or H category in contrast to the modal H/S grouping prevalent in speciments from L223. Occurrence of small numbers of softer individuals, even in reference lakes, suggests that at the time of these collections some crayfish had not reached their final overwintering state of exoskeletal hardness. To avoid this problem, in 1980 collections were delayed until one week prior to freeze-up and taken over a shorter time span

TABLE 2. Relationship of subjective carapace rigidity index to carapace ratio (carapace dry wt (mg)/unit length) and carapace punch (area = 33.2 mm^2) dry wt (mg) in *O. virilis* from ELA. Standard errors about the mean averaged ±0.26 for the carapace ratios and ±0.32 mg for the punch dry weights.

	L239 $n = 43$		L240 $n = 37$		L224 $n = 38$		L223 $n = 55$	
Index	Carapace ratio	punch dry wt	Carapace ratio	punch dry wt	Carapace ratio	punch dry wt	Carapace ratio	punch dry wt
VH	4.04	5.71	4.89	5.84	3.35	4.79	3.32	4.44
H	3.20	5.01	3.44	5.70	3.00	4.32	3.15	3.58
H/S	2.50	4.34	2.15	4.74	2.89	4.29	2.69	3.72
S/H	2.35	4.14	1.65	4.19	2.65	3.99	2.12	3.22
S	1.24	2.67	1.32	3.14	1.17	3.02	1.08	1.97

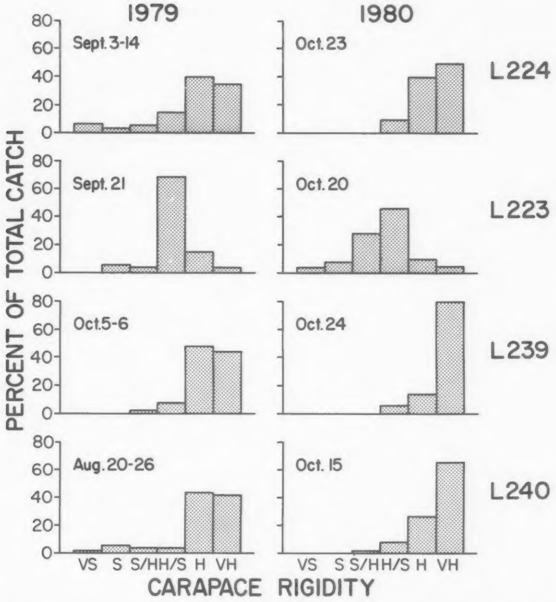

FIG. 1. Frequency distribution of subjective carapace rigidity for *O. virilis* in acidified L223 and three reference lakes. Scale ranges from very soft (VS), soft (S), intermediate soft-hard (S/H), and hard-soft (H/S), hard (H), to very hard (VH). Sample sizes were between 193 and 294 for all collections.

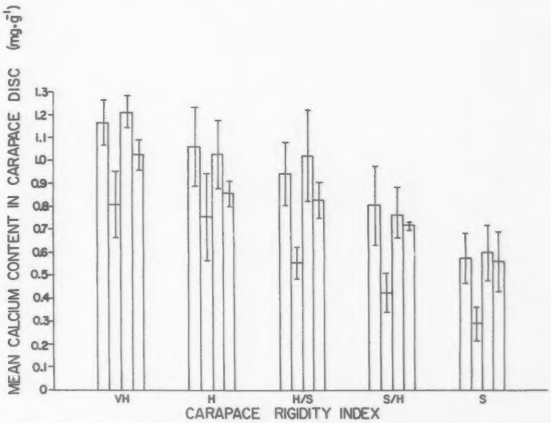

FIG. 2. Comparison of mean calcium content (± 95% C.I.) related to carapace rigidity index among ELA study lakes. Groupings for each index set arranged in order of decreasing lake calcium concentration: L239–L223–L240–L224. Sample sizes are the same as in Table 2.

to facilitate inter-lake comparisons. Most crayfish in the reference populations had attained the VH level by this time, while individuals in L223 formed a mode about the S/H–H/S divisions. The difference in carapace rigidity between the L223 and reference populations was not simply due to slower exoskeletal hardening as carapaces in L223 were still soft in the next year's spring collections. The proportion of animals with lost chelae or undergoing limb regeneration was higher in L223 (6.7%) than in reference lakes (L224 — 1.5%; L240 — 2.1%; L239 — 2.7%).

For speciments from each lake there was a correlation between both the mean carapace dry wt to length ratio or the dry wt of carapace disc, and the index of exoskeleton rigidity (Table 2). This indicated that subjective division of populations into hardness classes had some structural basis. An among-lake comparison showed that the dry wts of L239 and L240 carapaces were similar, while the L224 carapaces in the upper rigidity classes tended to weigh less, and those from L223 were almost always lowest in mass.

There were strong correlations between carapace disc dry wt and Ca content for each lake (France 1983). From these data the Ca content ($\bar{x}$ ± 95% C.I.) for each index level of carapace rigidity was calculated (Fig. 2). In all lakes there was a decrease in Ca content with progression from VH to S rigidity levels. Calcium content was significantly lower (ANOVA, LSD test, $P < 0.05$) in VH L224 individuals than in VH L239 and L240 animals. Values of Ca content were also significantly lower (ANOVA, $P < 0.001$) in L223 crayfish compared to those from reference lakes at every level of carapace rigidity.

Individual measurements of carapace disc Ca^{++} were expressed for each population on a mean percentage dry wt basis. Carapace Ca content was significantly lower (*t*-test, $P < 0.05$) in L223 crayfish ($\bar{x}$ % dry wt ± SE = 13.90 ± 0.54) than in animals from any of the three reference lakes (19.82 ± 0.33; 20.34 ± 0.63; 22.18 ± 0.51).

Individuals of *O. virilis* at ELA do not achieve the same degree of hardness as do individuals from harder waters (Malley 1980). If the assumption is made that there is a relationship between Ca content of crayfish exoskeletons and the Ca concentration of the lake water they inhabit, then the

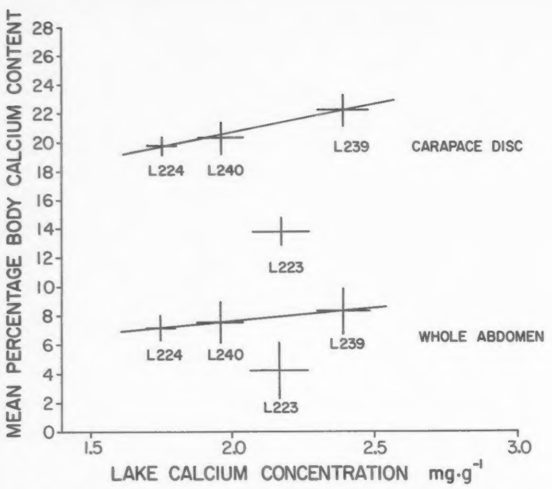

FIG. 3. Plot of mean percentage calcium content of carapace (n = 37–55) and whole abdomen (n = 4) preparations against average lake calcium concentration. Vertical and horizontal lines denote 95% confidence limits. Lake chemistry data from Prokopowich (1979).

TABLE 3. Abdominal muscle trace metal concentrations in $\mu g \cdot g^{-1}$ dry wt (average of 2 replicate determinations) for a composite sample of 20 to 30 adult *O. virilis* from each ELA study lake.

Lake	Mn	Al	Zn	Cu	Hg	Cd	Pb	Se
L223	240	46	74	79	0.52	0.10	3.8	1.6
L224	33	37	66	77	0.20	0.22	1.1	2.1
L240	70	58	69	66	0.36	0.12	1.7	1.3
L239	41	52	48	64	0.24	0.15	3.1	0.86

expected Ca^{++} concentrations of carapaces of L223 crayfish in the absence of acidification can be estimated, and the additional effect of acidification can be derived. From this it was calculated that crayfish in L223 have 35% less carapace Ca^{++} than background levels for the ELA region (Fig. 3). To compensate for small samples sizes of 37 to 55 for the Ca content determinations, the weighted mean population exoskeletal Ca content was estimated by multiplying the percentage values from the rigidity distribution (Fig. 1), based on sample sizes of between 193 and 294, with the results in Fig. 2: L224 — 20.57%; L239 — 20.59%; L240 — 20.07%; and this was compared with the L223 value — 14.77%, about 25% lower than the reference populations. Measurements of Ca concentration performed on whole abdomens used for trace metal analysis provide further evidence that L223 crayfish have significantly less body Ca than animals from reference lakes (Fig. 3).

Trace Metal Accumulation

Five of the seven metals had their highest concentration in L223 crayfish while four of the seven were lowest in the L224 sample (Table 3). A Friedman test (Hollander and Wolfe 1973) was performed on ranked data and indicated that there was no overall significant difference (t = 5.07, d.f. = 6, $P > 0.05$) in the ordering of tissue metal concentrations among lakes. Individually, however, L223 crayfish have accumulated higher levels of both Mn (L223 value of 240 $\mu g \cdot g^{-1}$ dry wt compared to a mean ± SE for the reference populations of 48 ± 11 $\mu g \cdot g^{-1}$ dry wt) and Hg (L223 value of 0.52 $\mu g \cdot g^{-1}$ dry wt compared to reference mean of 0.27 ± 0.05 $\mu g \cdot g^{-1}$ dry wt).

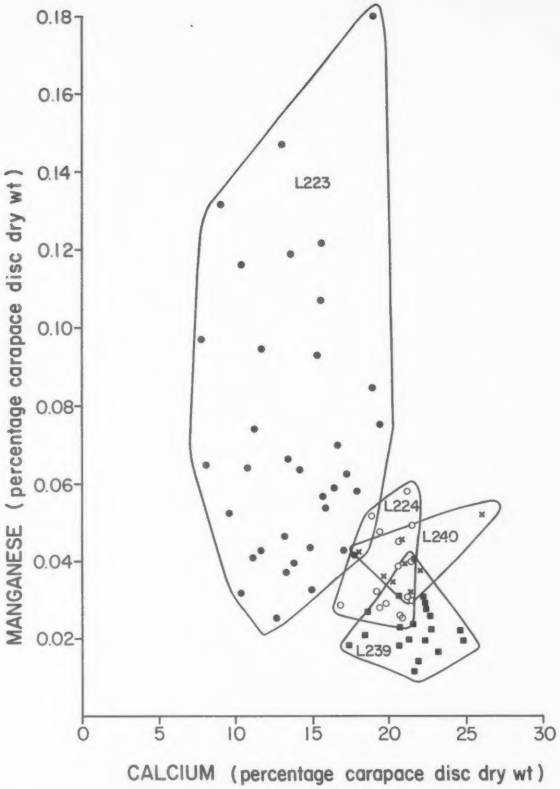

FIG. 4. Manganese and calcium content of *O. virilis* carapaces collected from ELA lakes during 1980.

Animals from L223 displayed a large variation in carapace Mn content (Fig. 4). No correlations were found between exoskeleton rigidity and Mn concentration in crayfish from any lake. Concentrations of carapace Mn were threefold higher in *O. virilis* from L223 than in crayfish from reference lakes. The ratio of carapace Ca to Mn was decreased from approximately 1000:1 in reference animals to 230:1 in L223 crayfish.

Discussion

Calcium Content

Postmolt crayfish depend largely upon uptake of Ca^{++} from the aquatic environment to recalcify their exoskeletons because the amount stored in the gastroliths or taken up through feeding is insufficient (Adegboye et al. 1977). Postmolt Ca^{++} uptake was reduced 60% in *Austropotamobius pallipes* in the absence of external HCO_3^- Greenaway 1974), and was inhibited below pH 5.75 and completely blocked at pH 4.0 in *O. virilis* (Malley 1980). Based on the experimental data in Fig. 2 in Malley (1980), one can empirically predict that during 1979 the L223 population experienced a 13% inhibition in Ca^{++} uptake at a mean lake epilimnion pH of 5.6. Further acidification in 1980 to pH 5.4 caused a 30–35% reduction in calcification. The present findings from the field verify these laboratory predictions. The carapace Ca^{++} content as mean % dry wt ± SE for L223 intermolt crayfish was 16.8 ± 0.4 in 1977 (Malley 1980), significantly ($P < 0.05$) higher than the value 13.90 ± 0.54 recorded during 1980 in this study. The % dry wt of Ca in intermolt *O. virilis* from reference lakes was 20–23%, comparable to values of 20–25% previously recorded for this species (Travis 1963; McWhinnie et al. 1969). Other processes of decalcification (Morgan and McMahon 1982) or reduced sclerotization (Yonge 1932) may have also contributed to the reduced exoskeleton rigidity observed in L223 crayfish.

Trace Metal Content

The range of Ca concentrations in the ELA study lakes ($1.7–2.4\ mg \cdot L^{-1}$) is too narrow to explain differences in metal uptake (France 1981), suggesting that the elevated tissue concentrations of Mn and Hg observed in L223 crayfish are probably related to acidification.

Accumulation of Hg by *O. virilis* occurs directly from the water (Hamilton 1972a). Tissue concentrations of Hg in this study are similar to values previously recorded for *O. virilis* at ELA ($0.17\ \mu g \cdot g^{-1}$; Hamilton 1972b), and intermediate between concentrations for *O. virilis* from northern Manitoba ($0.04\ \mu g \cdot g^{-1}$) and from Clay Lake ($0.95–10.12\ \mu g \cdot g^{-1}$), a mercury polluted system near Dryden, Ontario (Verner 1972; Hamilton 1972a). It is possible that sample preparation at 100°C may have been severe enough to volatize some Hg so that the presented results are underestimates (P.M. Stokes, Univ. of Toronto, pers. comm.).

The uptake of Mn^{++} is due not only to surface adsorption but also to substitution for $CaCO_3$ in the structural layers of invertebrates (Bryan and Ward 1965) as well as the ossified body parts of mammals (Kato 1963; Koshida et al. 1963). This is consistent with the geochemistry of Mn/Ca interactions (McBride 1979) and the chemical competition between these two divalent ions in plants (Ouellette and Dessureaux 1958; Sutton and Hallsworth 1958; Foy 1973). Lockhart and Lutz (1977) suggested that the large increases in Mn^{++} observed in skin and scales of George Lake white suckers may have been due to an impairment in Ca^{++} uptake as a result of competition for active cellular binding sites between the two elements. Although Mn was elevated in L223 exoskeletons coincident with lower Ca^{++} contents, this did not appear to be a process of simple substitution. Fraser and Harvey (1982) reached the same conclusion regarding the bone composition of white suckers. Further work is needed to discriminate possible subtle effects of Mn interference on Ca^{++} metabolism.

Potential Ecological Consequences

Limitation of calcification or other hardening mechanisms producing prolonged periods of soft exoskeletons could make populations more vulnerable to mechanical damage and cannibalism, both of which can limit crayfish distribution and production in softwater environments (Abrahamsson and Goldman 1970; Mills et al. 1976; Bretonne et al. 1969). Because adult crayfish are preyed upon by fish following ecdysis when exoskeletons are still soft and palatable (Scott and Duncan 1967; Stein 1977), they remain in seclusion (Mobberly 1967; Stein 1977), a behavioural trait absent in intermolt crayfish with reduced carapace rigidity caused by acidification. As a result of this, predation could have contributed to the increased population mortality rates observed by France and Graham (1985) for crayfish in L223. Imbalance in Ca^{++}uptake may also eventually disturb the molting cycle (Borgstrom and Hendrey 1976; Malley 1980; Appelberg 1980) and affect growth and onset of sexual maturity. Contrary, however, to implications from laboratory studies, the timing of molting events and growth rate of L223 crayfish had not been altered by acidification to pH 5.1 in 1981 (France 1985). The higher proportion of crayfish in L223 with missing chelae compared to reference populations may reflect increased antagonism among softer animals, or may indicate an inhibition of limb regeneration at low pH (Needham 1947). Chelae serve an important intraspecific behavioral and ecological role (Bovberg 1953), as well as deterring predation (Stein 1977).

Information on Mn toxicity to aquatic organisms, particularly in acidified systems (Anderson and Nyberg 1984), is rare. Despite some data implying that crayfish may be able to tolerate Mn concentrations at least as high as $1000–2000\ \mu g \cdot L^{-1}$ (Water Quality Criteria 1972), its toxicity is known to depend on a number of other factors including interaction with other elements such as Ca (Chandra et al. 1974; Gale et al. 1973) or Fe and Al (Anderson and Nyberg 1984). The content of Mn in acidified Swedish lakes ranges from 200 to 400 $\mu g \cdot L^{-1}$ (Dickson 1975), much higher than the concentration of $100\ \mu g \cdot L^{-1}$ suggested to exert hazardous sublethal effects (Water Quality Criteria 1972). For example, Boutet and Chaisemartin (1972) found that Mn diminished crayfish reproductive capacity by half at concentrations only 40–50% of those producing 30 d LC50's. Such chronic effects of Mn in acid water remain unknown.

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Responses of Fish Populations in Lake 223 to 8 Years of Experimental Acidification

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Lake 223 in the Experimental Lakes Area, northwestern Ontario, was experimentally acidified with sulfuric acid from 1976 (initial average pH 6.49) to 1981 (average pH 5.02), and then maintained at pH 5.02 to 5.13 from 1981 to 1983. Lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), fathead minnow (*Pimephales promelas*), and slimy sculpin (*Cottus cognatus*) were abundant at the onset of acidification. A decline in the abundance of fathead minnow began in 1979 (pH 5.64), and was followed first by a rapid increase in number of pearl dace (*Semotilus margarita*) in 1980 (pH 5.59) and then a rapid decrease in 1982 (pH 5.09). The abundance of slimy sculpin decreased rapidly in 1979. Abundances of lake trout and white sucker increased during the early years of acidification, but declined following consecutive recruitment failures starting in 1980 for trout and in 1981 for white suckers. By 1982 recruitment had ceased for all Lake 223 fishes. Survival of lake trout ≥ age 1 decreased in 1982 and 1983, but no other changes in survival of fish ≥ age 1 were detected for lake trout or white sucker. By spring 1983 many lake trout were emaciated due to losses of the lake trout food organisms. No changes in growth of lake trout and white suckers occurred during the initial years of acidification, but growth of lake trout slowed in 1982 and growth of white sucker increased in 1979.

Le lac 223 de la Zone de lacs expérimentaux du nord-ouest de l'Ontario a fait l'objet d'une acidification expérimentale à l'acide sulfurique de 1976 (pH de départ moyen de 6,49) à 1981 (pH moyen de 5,02). Les eaux du lac ont ensuite été maintenues à un pH moyen de 5,02–5,13 de 1981 à 1983. Le touladi (*Salvelinus namaycush*), le meunier noir (*Catostomus commersoni*), le méné tête-de-boule (*Pimephales promelas*) et le chabot visqueux (*Cottus cognatus*) étaient abondants au début de la période d'acidification. Un déclin de l'abondance du méné tête-de-boule a été noté en 1979 (pH de 5,64). Il a été suivi d'une augmentation rapide de l'abondance du mulet perlé (*Semotilus margarita*) en 1980 (pH de 5,59) elle-même suivie d'un déclin rapide en 1982 (pH de 5,09). Le chabot visqueux est rapidement devenu moins abondant en 1979. Le touladi et le meunier noir sont devenus plus abondants au début de la période d'acidification, mais ces poissons sont devenus moins abondants suite à des échecs consécutifs du recrutement qui ont débuté en 1980 pour le touladi et en 1981 pour le meunier noir. En 1982, le recrutement avait cessé chez toutes les espèces. Le taux de survie des touladis d'âge ≥ 1 a diminué en 1982 et 1983, mais aucune autre modification du taux n'a été notée pour les touladis et les meuniers d'âge ≥ 1. Au printemps de 1983, bon nombre de touladis étaient émaciés suite à la disparition d'organismes leur servant de nourriture. Aucune modification du taux de croissance des touladis et des meuniers noirs n'a été notée au cours des premières années de l'acidification, mais le taux de croissance des touladis a ralenti en 1982 et celui des meuniers noirs s'est accru en 1979.

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The scientific literature on adverse effects of lake acidification for fish populations has rapidly expanded in recent years. These publications are often based on either surveys or single species toxicity tests. In surveys, the losses of fish populations, determined by fish netting or creel census, are often coupled with less frequent pH measurements to derive toxic pH thresholds and to quantify the loss of fish populations (Harvey and Lee 1982; Schofield 1982; Minns 1981; Muniz and Leivestad 1980; and others). In toxicity tests, individuals are held in the laboratory or in situ for studying effects of acidification on specific tissues or determining toxic pH thresholds (and in some instances toxic metal and pH thresholds) on different life stages (Spry et al. 1981; Fromm 1980; Baker 1982). Both approaches have weaknesses. In the former, the inferred toxic threshold may be confounded by factors such as the longevity of some species or vulnerability to fishing gear. In the latter, length of exposures may be unrealistically short, or the interactions with other species ignored. Most studies have concentrated on the responses of single species that are important to sport or commercial fisheries, rather than on responses of all fish species in a lake community.

Starting in 1976, Lake 223 in the Experimental Lakes Area (ELA), northwestern Ontario, was progressively acidified with sulfuric acid to quantify the relationship between acid input and responses of the physical, chemical, and biotic components of a small lake (Schindler 1980; Schindler et al. 1980; Malley et al. 1982; Schindler and Turner 1982; Schindler et al. 1985; Shearer et al. 1987; France 1987). In this paper we describe effects of the first 8 yr of acidification (1976–83) on the fish populations of Lake 223. The pH thresholds for toxic effects on the fish populations are compared to previous labo-

TABLE 1. pH of six lakes in the Experimental Lakes Area. Values for lakes 223 and 302 are open-water means for epilimnetic data from 1974 to 1983. The 1973 samples were taken at 3 m. The 1978 and 1982 samples were taken at $\approx$0.5 m

Lake	Year 1973[a]	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983
Lake 223 (actual)	7.00	6.70	6.64	6.49	6.13	5.93	5.64	5.59	5.02	5.09	5.13
(goal)	—	—	—	6.50	6.00	5.75	5.50	5.25	5.00	5.00	5.00
224	7.10	—	—	—	—	7.00[b]	—	—	—	6.82[b]	—
302	6.40	—	—	—	—	—	—	6.34	6.34	6.08	—
305	7.40	—	—	—	—	6.91[b]	—	—	—	7.12[b]	—
382	6.80	—	—	—	—	6.83[b]	—	—	—	6.99	—
468	7.00	—	—	—	—	7.00[b]	—	—	—	7.09	—

[a] Beamish et al. 1976 (survey fall 1973).

[b] ELA staff, unpublished data (surveys falls 1978, 1982).

ratory studies. Data are also presented or summarized for fish populations in other unacidified ELA lakes (denoted as "reference populations").

Changes in fish recruitment, growth, and mortality have been identified in acidified lakes (Haines 1981). Decreased recruitment, usually due to increased mortality of eggs or fry (Peterson et al. 1982), has been implicated as a primary mechanism for the decline of fish populations. Although changes in fish growth (or condition) have been used as sensitive indicators of the status of a fish population (Weatherley 1972; Nikolsky 1963), growth of fishes in acid lakes may either increase (e.g. Ryan and Harvey 1977) or decrease (e.g. Beamish 1974). The condition factor (ponderal index) may increase for fish populations in acid lakes (Harvey 1982; Rosseland et al. 1980) or be unrelated to pH status (Wiener and Hanneman 1982). In addition to increased egg and larval mortality, increased mortality of adult fish has been documented for stream-dwelling populations (Leivestad and Muniz 1976) and inferred for lacustrine populations (Beamish and Harvey 1972). Consequently, we monitored recruitment, growth, condition, and adult survival as intensively as possible to determine causal mechanisms of population decline and to evaluate their potential as indicators of population stress.

Materials and Methods

Study Lakes

Lake 223 — Lake 223 is a small (27.4 ha, z_{max} 14.4 m) lake in the ELA. It stratifies during summer months and is typical of small lake trout (*Salvelinus namaycush*) lakes in northwestern Ontario (Beamish et al. 1976; Martin and Olver 1976). Electrolyte grade sulfuric acid was added to Lake 223 from 1976 to 1983 to progressively lower the average epilimnetic pH from 6.64 in 1975 to 5.13 in 1983 (Table 1). Details of the acidification are located in Cruikshank (1984) and changes in water chemistry for the first 4 yr of acidification are located in Schindler et al. (1980). Elevated aluminum levels have often occurred in acidic lakes (Baker 1982; Leivestad 1982; and others). Total aluminum concentrations in Lake 223 only increased from $\simeq 10\ \mu g \cdot L^{-1}$ to $\simeq 40\ \mu g \cdot L^{-1}$ during the 8 yr of acidification, well below toxic levels reported in other studies.

Seven fish species were present in Lake 223 at the start of acidification: lake trout, white sucker (*Catostomus commersoni*), fathead minnow (*Pimephales promelas*), pearl dace (*Semotilus margarita*), finescale dace (*Phoxinus neogaeus*), brook stickleback (*Culaea inconstans*), and slimy sculpin (*Cottus cognatus*). This assemblage is typical of many small (<40 ha) lake trout lakes in the ELA (Beamish et al. 1976), although the number of cyprinid species that are present varies among lakes.

Reference lakes — Lake trout and white sucker populations were monitored in five unacidified ELA lakes — Lakes 224, 302, 305, 382, and 468 (Cleugh and Hauser 1971 summarized locations and physical characteristics of these lakes). The primary reference lakes were Lakes 224 (lake trout) and 302 (white sucker). Because Lake 302 was used for an acidification experiment commencing during summer 1982, only data collected prior to summer 1982 were used for comparisons to Lake 223 data.

Fathead minnow, pearl dace, finescale dace, and slimy sculpin populations were not monitored as intensively as lake trout and white sucker populations in ELA reference lakes. Cyprinid populations were monitored in Lakes 114 (1978–83), 303 (1979, 1983), and 302 (1979–81). Incidental catches of these species were also noted, but not quantified, in Lake 224 (1976, 1978–83). Small amounts of sulfuric acid were added to Lake 114 from 1979 to 1983 but the average epilimnetic pH did not change appreciably (5.83 in 1979, 5.88 in 1983).

Lake Trout

Lake trout from Lake 223 were captured, sampled, and released each spring and fall from fall 1975 to fall 1985 using trap nets, gill nets, and angling. The same methods were used to sample lake trout each autumn in Lake 224 in 1976 and 1978–85. Small-mesh (6.4 mm) deepwater and shallow-water trap nets, similar to those described by Beamish (1973), were fished along lake shorelines each spring and fall. Fish were captured in spring or fall when water temperatures were less than 14°C. One or more small-mesh multifilament gillnets (12.5-, 16-, 19-, or 22-mm bar mesh) were fished at dusk and emptied every 15–30 min to minimize fish mortality. Angling was conducted with heavy monofilament line (7.7 kg test) and barbless lures; fish were quickly netted after striking the lures (<5 min). All fish caught by gillnetting or angling were held overnight in pens prior to further sampling to assess mortality due to these capture methods (5.1% for gillnetting, 4.7% for angling). While no fish was intentionally sacrificed to obtain stomach contents, stomachs were examined from fish that died during capture. In addition, prey items that were regurgitated after capture by angling or gillnetting were also noted.

All lake trout were anesthetized with MS-222® (tricaine-methane-sulfonate), weighed to the nearest gram, measured to the nearest millimetre (fork length), and batch marked by scarring soft fin rays on the dorsal or anal fin as described in Welch and Mills (1981). Trout larger than 37 cm were tagged with individually numbered Carlin tags (White and Beamish 1972).

Lake trout spawning areas in Lakes 223 and 224 were located in 1979, and were observed during succeeding years to assess spawning activity. The entire shorelines of each lake were examined at night during spawning to identify changes in spawning locations from 1979 to 1983. In addition, a few female trout (≤ 5) from each lake (Lakes 223, 224, 305, and 468) were sacrificed yearly (1977–83), approximately 2–3 wk after spawning had ceased, to determine if Lake 223 females were resorbing an unusually large number of eggs.

All lake trout from Lakes 223, 224, 382, 305, and 468 were aged by examining pectoral or pelvic fin-ray sections (Cuerrier 1951). Fish from Lakes 382, 305, and 468 were captured using multipanel gillnets (11-, 25-, 30-, 33-, 38-, and 45-mm bar mesh). The fin-ray method was validated for Lake 223 lake trout using mark–recapture methods similar to those used by Mills and Beamish (1980). Mean length at age data were calculated each fall, 1975 to 1983. Condition factors (k) were calculated for 1975–83 data using $k = 100w/l^3$ (Bagenal and Tesch 1978) where w is weight (g) and l is fork length (mm).

The Jolly–Seber "full" model (Jolly 1965; Seber 1982) was used to estimate abundance ($\hat{N}_i$) of lake trout ($\geq$ age 1) in Lakes 223 and 224 for all but the first sampling period in each lake. No estimates for age 0 fish were made. Abundance estimates for the first sampling periods (fall 1975 in Lake 223 and fall 1976 in Lake 224) were obtained using the "death-only" model after stratifying capture histories so that new recruits (based on age of lake trout) were excluded. The number of new recruits at age 1 ($\hat{B}_i$) entering the trout populations each year and annual survival ($\hat{\phi}_i$) between successive fall samples were estimated using the Jolly–Seber full model (spring samples were omitted). Recruitment of individuals in Lake 223 was also estimated from 1976 to 1983 based on catches of individual year-classes. The program package of Arnason and Baniuk (1978) was used to derive $\hat{N}_i$, $\hat{B}_i$, and $\hat{\phi}_i$. Commencing in 1979, sample sizes for fall sampling periods were based on simulation results (using Arnason and Baniuk 1978) that suggested $\hat{N}_i$, $\hat{\phi}_i$, and $\hat{B}_i$ would be relatively precise ($\pm 10\%$ confidence interval).

White Sucker

White suckers were captured, sampled, and released in Lake 223 with trap nets and gill nets from fall 1975 to fall 1985 during each spring and fall. In addition, fish were trapnetted during summer 1978–83. After capture, white suckers were sampled similarly to Lake 223 lake trout and aged by examining pectoral and pelvic fin rays (Chalanchuk 1984). Age determinations were validated by mark–recapture and length frequency methods (Chalanchuk 1985). Mean length at age data were calculated each spring, 1976 to 1983. Back calculations (Chalanchuk 1985) were used to construct growth curves for 1973 to 1975; these were plotted and lines were drawn by eye through the upper and lower confidence limits to form a range of growth to compare to later years. Abundance ($\hat{N}_i$), annual survival ($\hat{\phi}_i$), recruitment ($\hat{B}_i$), and condition factors (k) of white suckers were calculated as described for lake trout. Condition factors were also calculated for white suckers from Lakes 302 and 468. Too few white suckers were captured during fall 1975 to form an abundance estimate for Lake 223, so the first sampling period was spring 1976. Estimates of annual survival ($\hat{\phi}_i$) and new recruits($\hat{B}_i$) were calculated between successive spring samples.

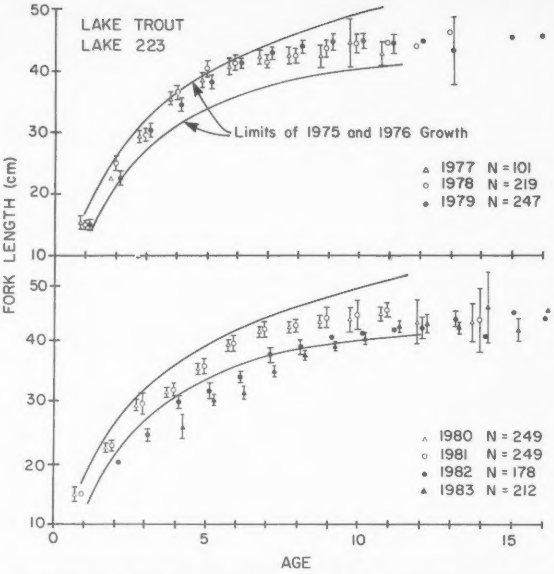

FIG. 1. Growth data for lake trout in acidified Lake 223 from 1977 to 1983. Mean lengths and 95% confidence intervals are presented. The range of lake trout growth (drawn from means and 95% confidence intervals for age-groups), for 1975 (N = 55) and 1976 (N = 110), prior to lake pH decline, is also presented in both panels.

Other Species

Slimy sculpins in Lake 223 were visually censused by SCUBA diving at night along line transects in early September from 1976 to 1982. Abundance estimates per unit area were calculated from 1978 to 1981 data following the methods of Davies (I. J. Davies, Freshwater Institute, unpublished data).

Fathead minnow abundance was estimated with small-mesh (3 mm) trap nets in Lake 223 after one night sets in August 1976 and 1978. Fish were counted and measured to the nearest millimetre (fork length). All individuals caught in trap nets from July to September 1979 were counted, measured, and released. Length-frequency distributions were constructed for the 1976, 1978, and 1979 catches. Fathead minnows were caught very rarely from 1980 to 1983, despite increased sampling effort.

Pearl dace were caught rarely in Lake 223 prior to 1979. During July to August in 1980 and 1981 pearl dace were collected from one night trapnet sets, counted, and measured to the nearest millimetre (fork length). In 1983, all individuals caught in trap nets throughout the ice-free season were counted, measured, and released. Length-frequency distribu-

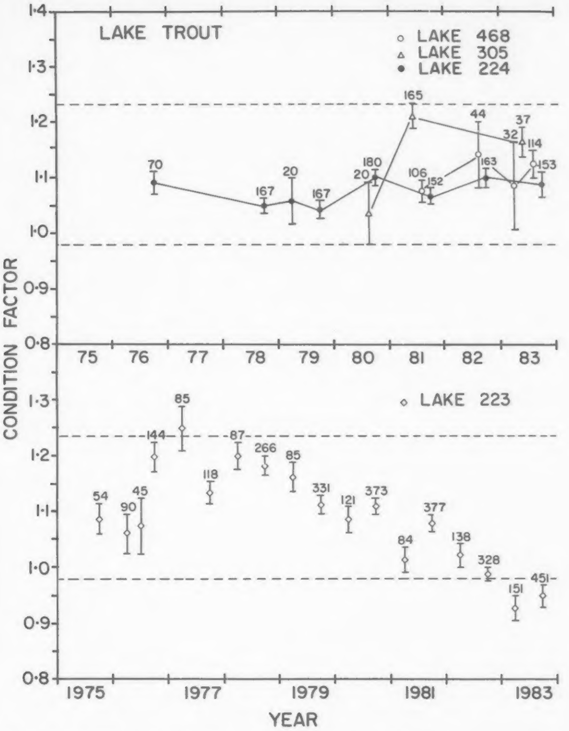

FIG. 2. Condition of lake trout from reference Lakes 224, 305, and 468, and acidified Lake 223. Means, 95% confidence intervals, and sample sizes are presented. Dotted lines represent the extremes of upper and lower confidence limits of condition for reference Lakes 224, 305, and 468.

tions were prepared for the 1980−82 catches. Few pearl dace were caught in 1983.

Pearl dace and fathead minnows were collected in one night sets of trap nets in (1) Lake 114 from 1978 to 1983, (2) Lake 303 in 1979 and 1983, and (3) Lake 302 from 1979 to 1981. Netting was usually conducted monthly during each open-water season. Individuals were measured to the nearest millimetre and length-frequency distributions were constructed in the same manner as those for Lake 223.

Finescale dace and brook sticklebacks were caught rarely in Lake 223. Fewer than 20 finescale dace from 1975 to 1980 and 10 sticklebacks were caught each year from 1975 to 1983. Individual lengths for each species were recorded, but small sample sizes precluded analyses of these data. No finescale dace were caught after 1980.

Results

Lake Trout

Growth data for lake trout in Lake 223 were very similar for 1975 (pH 6.64) and 1976 (pH 6.49). As the pH of Lake 223 declined from 6.13 to 5.02 during 1977−81, lake trout growth remained similar to 1975 and 1976 (Fig. 1). Growth of 2−6-yr-old fish decreased significantly in 1982 compared to previous years, and declined further in 1983. In general, lengths of individual trout did not change from 1982 to 1983.

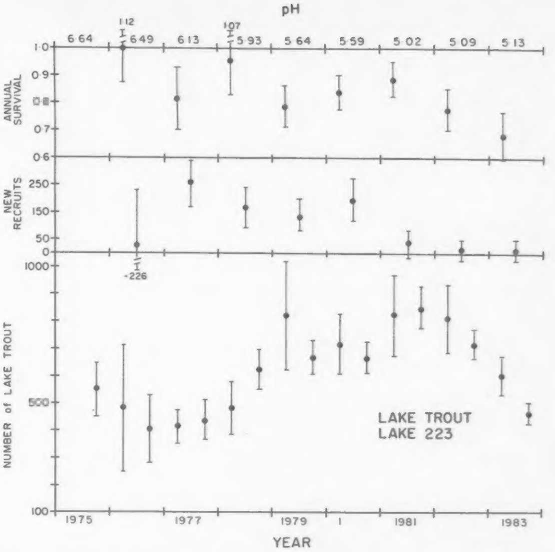

FIG. 3. Abundance ($\hat{N}_i$), recruitment ($\hat{B}_i$), and annual survival ($\hat{\phi}_i$) of lake trout (≥ age 1) in acidified Lake 223. Estimates and 95% confidence intervals are plotted.

Condition (k) of Lake 223 trout increased from fall 1975 to spring 1977 (Fig. 2), when trout were in better condition than at any time in Lakes 224, 305, and 468. Condition of lake trout in Lake 223 gradually declined from 1977 (pH 6.13) to 1983 (pH 5.13). In 1983, trout condition in Lake 223 was less than at any time in any reference lake. Most lake trout, regardless of size, were robust during fall 1976 to 1978, but many were very emaciated by fall 1982. Even though most trout were in very low condition during fall 1982 to fall 1983, a few large fish (>550 mm) remained in fairly good condition. Condition of lake trout in Lakes 224 (1976−83) and 468 (1981−83) varied little from year to year, while condition in the Lake 305 population increased from 1980 to 1983.

Abundance ($\hat{N}_i$) of Lake 223 trout decreased slightly from fall 1975 to fall 1976, but then increased to reach approximately the 1975 level by fall 1977 (Fig. 3). The poor precision of the spring 1976 $\hat{N}_i$ was due to the relatively small sample size of the catch for this sampling period. The population steadily increased (≈240%) from 1976 to 1981, and then declined (≈50%) from fall 1981 to fall 1983. In contrast, abundance of Lake 224 trout (Fig. 4) was relatively stable from 1976 to 1983. The estimates for fall 1976 and fall 1978 had poor precision (±40%) relative to other years. There is usually a positive correlation between the size of an individual $\hat{N}_i$ and its confidence interval (Manly 1971), so the relatively larger $\hat{N}_i$ for 1976 and 1978 compared to estimates for 1979−82 are not surprising when the relatively larger confidence intervals of these estimates are considered.

Annual survival ($\hat{\phi}_i$) of Lake 223 trout was relatively stable from 1976 to 1981, but decreased from 1981 to 1983 (Fig. 4). The survival estimates for 1976 and 1978 are somewhat higher than other estimates from 1977 to 1980, but had poor precision. Again, there is a correlation between the size of the confidence interval and size of an estimate. Therefore, the higher $\hat{\phi}_i$ for 1976 and 1978 probably reflect poor precision rather than higher survival. The average annual survival from

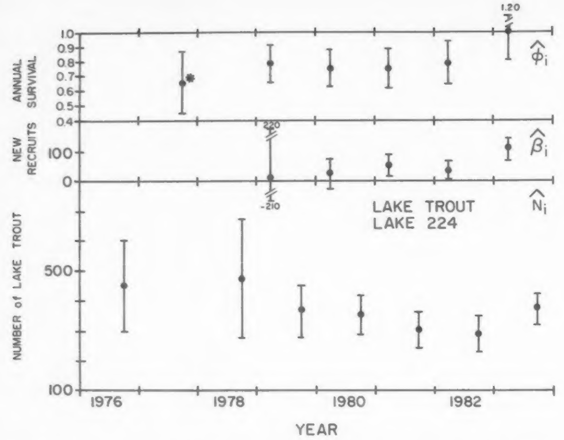

FIG. 4. Abundance ($\hat{N}_i$), recruitment ($\hat{B}_i$), and annual survival ($\hat{\phi}_i$) of lake trout (≥ age 1) in reference Lake 224. Estimates and 95% confidence intervals are plotted. The asterisk identifies a $\hat{\phi}_i$ for a 2-yr period. All other $\hat{\phi}_i$ are for 1 yr.

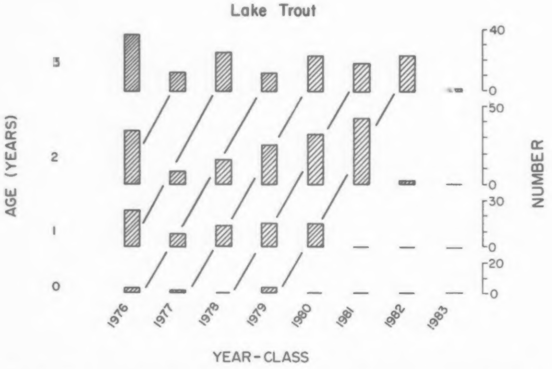

FIG. 5. Total catch of individual year-classes from age 0 to age 3 of lake trout in Lake 223.

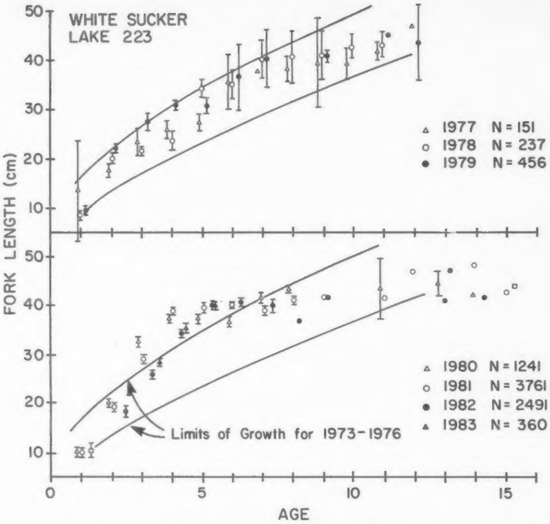

FIG. 6. Growth data for white sucker in acidified Lake 223 from 1977 to 1983. Mean lengths and 95% confidence intervals are presented. The range of white sucker growth for years prior to lake pH decline (1973, N = 135; 1974, N = 178; 1975, N = 194; and 1976, N = 550), drawn from means and 95% confidence intervals for age-groups, is also presented.

1976 to 1981 was 87%·yr^{-1}. The average annual survival for Lake 224 trout from 1976 to 1983 was 83%·yr^{-1} (Fig. 4). The 1977 estimate is the average survival for the 2-yr period from 1976 to 1978; one takes the square root of this $\hat{\phi}_i$ to obtain individual estimates for each year (this gives estimates of 81% ·yr^{-1}). The $\hat{\phi}_i$ for 1983 is very high, due to poor precision. As more data are collected, this estimate will probably be lower. Annual survival of Lake 223 trout (1976–81) was similar to annual survival for Lake 224 trout (1976–83). The decrease in survival of Lake 223 trout (1981–83) did not occur in the Lake 224 population.

The entry of new recruits ($\hat{B}_i$, age 1 fish) into the Lake 223 trout population was highest in 1977 (the 1976 year-class) and 1980 (the 1979 year-class, Fig. 3). Recruitment was slightly lower in 1978 and 1979. The $\hat{B}_i$ for 1976 was very imprecise and difficult to interpret, due to small sample sizes in 1975 and 1976. The catch-at-age data (Fig. 5) showed there was successful recruitment in 1976 and that it was similar to recruitment for the years 1977 to 1980. The 1981 to 1983 $\hat{B}_i$ were very low, not significantly different from zero recruitment; these agree well with catch data for the 1980 to 1982 year-classes (Fig. 5). The year-class failure in 1983 (Fig. 3) would not be apparent in $\hat{B}_i$ until 1984 estimates are available. The entry of 1-yr-old recruits into the Lake 224 (Fig. 4) population varied little between years, except for higher recruitment in 1983. The estimate for 1979 was hard to interpret because of extremely poor precision (due to the relatively small sample size in 1978). Recruitment was relatively constant, but at a low yearly level. Lake trout successfully spawned during each year of study (1979–83) in Lake 223. There was no indication for any year that female lake trout in Lake 223 were resorbing more eggs than those in Lakes 224, 305, and 468. Lake trout utilized the same spawning beds in Lake 223 from 1979 to 1981, but utilized different spawning areas each year in 1982 and 1983. Lake trout utilized the same spawning beds in Lake 224 from 1979 to 1983.

Stomach contents from dead trout and regurgitated contents from fish caught by angling or gillnets varied between years. In 1977 and 1978, trout often regurgitated fathead minnows, while *Mysis relicta* were also present in stomachs that were examined. In 1979, pearl dace and fathead minnows were often regurgitated; white sucker and slimy sculpins were also present in some of the stomachs of dead fish. From 1980 to 1981, lake trout often regurgitated small (<150 mm) white suckers or pearl dace; the stomachs of some smaller trout contained *Daphnia catawba*. Very few trout regurgitated food in fall 1982 and 1983; the few, very large trout (>3 kg) sometimes regurgitated smaller trout (<30 cm). Stomachs of trout that died during capture were always empty in 1983.

White Sucker

Growth of white sucker varied considerably throughout the acidification of Lake 223. Growth of white suckers in Lake

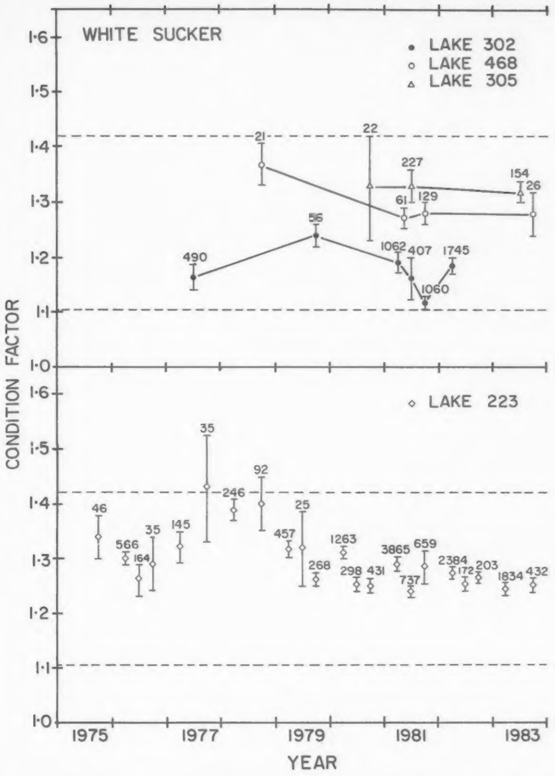

FIG. 7. Condition of white sucker from reference Lakes 302, 305, and 468, and acidified Lake 223. Means, 95% confidence intervals, and sample sizes are presented. Dotted lines in both panels represent the upper and lower limits of condition for reference lakes 302, 305, and 468.

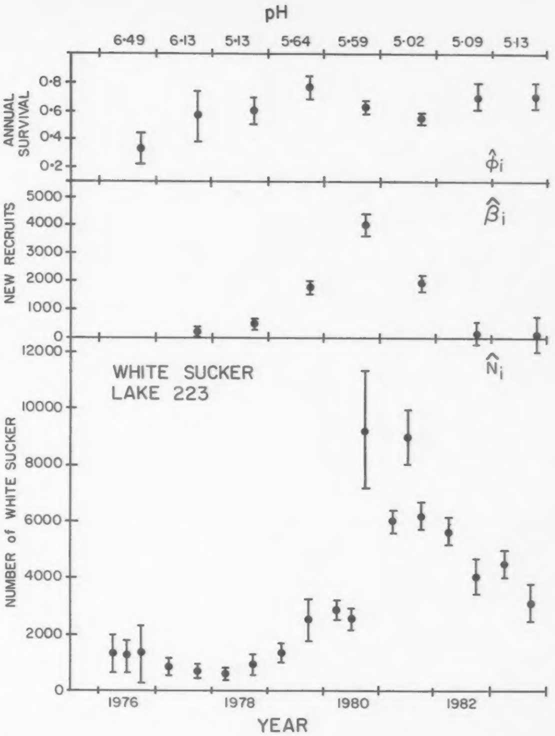

FIG. 8. Abundance ($\hat{N}_i$), recruitment ($\hat{B}_i$), and annual survival ($\hat{\phi}_i$) of white sucker (≥ age 1) for Lake 223. Estimates and 95% confidence intervals are plotted.

223 gradually increased for age classes 2–6 starting in 1979 (Fig. 6), and peaked in 1980 and 1981. In 1982 and 1983 growth of the age 2–6 fish declined. For a more complete synopsis of Lake 223 white sucker growth, see Chalanchuk (1985).

Condition of white sucker in Lake 223 increased from the start of the experiment to 1977 and 1978, declined in 1979, and then remained relatively stable from 1979 to 1983 (Fig. 7). Condition of Lake 302 (pH 6.4) white sucker increased from 1977 to 1979, decreased until fall 1981, and then increased in 1982. White sucker condition in Lake 468 decreased slightly from 1978 to 1981, and then remained relatively unchanged through 1983. White sucker condition in Lake 305 remained nearly constant during all years this lake was studied. Although white sucker condition in Lake 223 decreased during the later years of acidification, condition during 1983 remained substantially higher than that in Lake 302 and was only slightly lower than that in Lakes 468 and 305.

The abundance ($\hat{N}_i$) of white sucker in Lake 223 remained relatively constant from 1976 (pH 6.49) to 1978 (pH 5.3), increased 500–800% from 1978 to 1980–81 and then declined from 1981 to 1983 (Fig. 8). The population in fall 1983 was approximately 3× the population size at the start of the experiment in 1976.

Annual survival of Lake 223 white sucker increased gradually from 1976 to 1979, then declined from 1979 to 1981, and increased again in 1982 (Fig. 8).

Recruitment ($\hat{B}_i$) of white suckers at age 1 increased about 8× from 1977 (pH 6.13) to 1980 (pH 5.59) and then declined to 1982 (pH 5.09, Fig. 8). The 1982 and 1983 estimates are not significantly different from zero recruitment. The Jolly–Seber estimates of recruitment agreed well with the catch-at-age data for white sucker (Fig. 9); there were strong year-classes from 1976 to 1980, but extremely weak or completely missing year-classes from 1981 (pH 5.02) to 1983 (pH 5.13). There was no indication for any year that female white suckers in Lake 223 were resorbing more eggs than those in Lakes 224, 305, and 468. White suckers utilized the same spawning areas in Lake 223 each year.

Fathead Minnow and Pearl Dace

The fathead minnow was the most abundant of the cyprinids that were present in Lake 223 at the start of acidification. Catches declined from approximately 2 000 fish per net (overnight set) in 1976–78 to less than 1 fish per net in 1979 (Fig. 10). In 1979, 87% of the fish caught were greater than 5 cm in length (2–4-yr-old fish). In 1976 and 1978, only ≈11% of the catch was larger than 5 cm in length; most fish were 2–5 cm long (age 1). Age 1 fish (the 1978 year-class) were almost completely absent during 1979. Fewer than 30 fathead minnows were captured in 1980; all were 2–5 cm long. Fewer

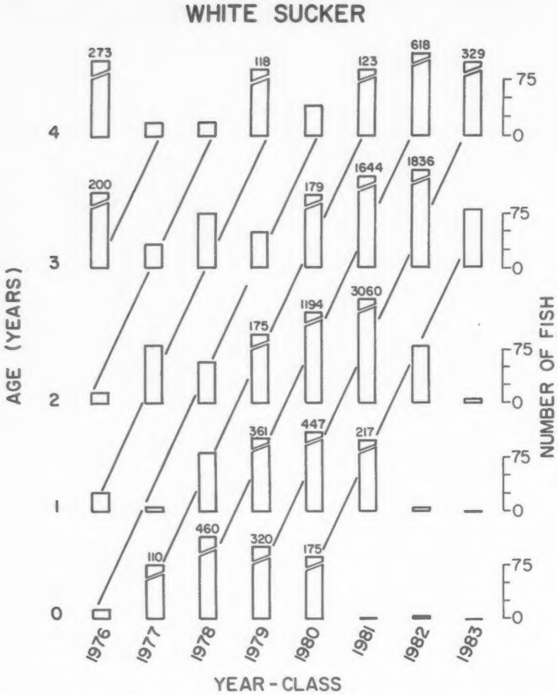

FIG. 9. Total catch of individual year-classes from age 0 to 4 of white sucker in Lake 223.

than 5 were captured in 1981 and none were captured after 1981.

Pearl dace were rare in Lake 223 at the start of this study. Few were captured in summer 1978, but the population increased dramatically in 1980 (Fig. 11). About 75% of the pearl dace caught in 1980 were less than 7 cm in length (age 0 fish). Pearl dace of all age-groups were abundant in 1981, but those less than 7 cm comprised only 5% of the catch in 1982 (Fig. 11); very few age 0 fish were present. In 1983 only a few pearl dace, all exceeding 8 cm, were captured.

Fathead minnow and pearl dace were abundant in Lake 114 from 1978 to 1983. Results for 1978 and 1979 are summarized in Tallman et al. (1984); they were similar to catches of fathead minnow in Lake 223 in 1976 and 1978 (Fig. 10), and pearl dace catches in Lake 223 in 1980 (Fig. 11). Fathead minnow catches from 1980 to 1983 in Lake 114 were similar to those in Lake 223 for 1978 and 1979, and Lake 114 pearl dace catches from 1980 to 1983 were similar to Lake 223 catches in 1980. Even though length-frequency data were not collected for Lake 224 fathead minnow and pearl dace, many were present in trapnet catches each year from 1978 to 1983. Abundant populations existed in each lake from 1978 to 1983. There were no indications of year-class failures or extreme fluctuations in abundances.

Slimy Sculpin

The number of slimy sculpin in Lake 223 decreased rapidly in 1979 (pH 5.64, Fig. 12). Sculpins were caught only occasionally in 1982 and none were caught in 1983. In general, fewer large and very small sculpins were seen after 1978 than in previous years. Slimy sculpins were present in trapnet catches each year, 1978–83, in Lake 224.

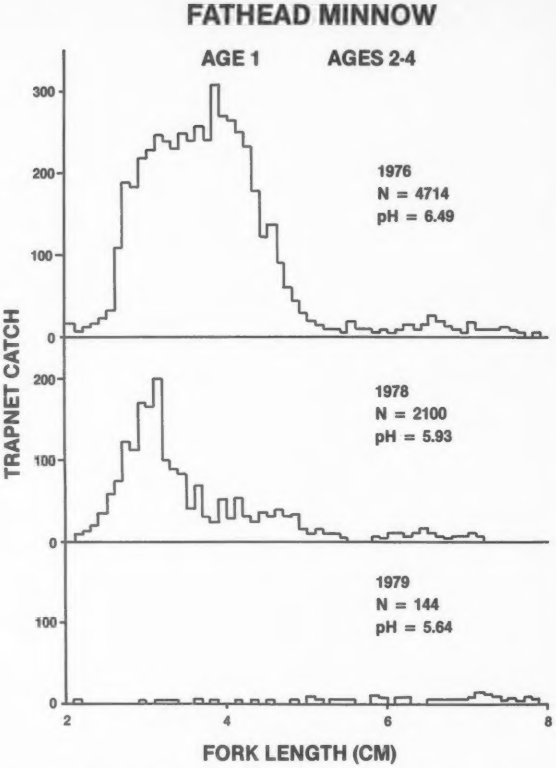

FIG. 10. Catch of fathead minnow in trapnets for 1976, 1978, and 1979. Catches for 1976 and 1978 are from one 24-h set of one net for each year. Catch for 1979 is the total number caught in 5 nets for 197 24-h sets.

Discussion

Thresholds for Population Decline

The primary mechanism for the population declines of lake trout, white sucker, fathead minnow, and pearl dace in Lake 223 was recruitment failure. The cause of slimy sculpin abundance decline was less clear. In general, pH thresholds for recruitment failures of Lake 223 species are similar to thresholds inferred from survey and laboratory results.

The threshold for lake trout recruitment failure in Lake 223 was pH 5.59 (Fig. 5). This pH is similar to thresholds inferred from surveys of lake trout presence and lake pH in New York Adirondack lakes (pH 5.49, Schofield 1982), in Ontario La Cloche Mountain lakes (pH 5.2–5.5, Beamish 1976), and in a more widespread central Ontario survey (pH 5.2–5.5, Beggs et al. 1985). Recruitment failures of age 0 lake trout in Lake 223 commenced in 1980 (pH 5.59, Fig. 5) and mortality of fish ⩾ age 1 increased in 1982 (pH 5.09, Fig. 3). Increased mortality of adult fish often has been implicated as a primary mechanism for population decline in acidic lakes (Beamish 1974; Muniz and Leivestad 1980), but the primary mechanism

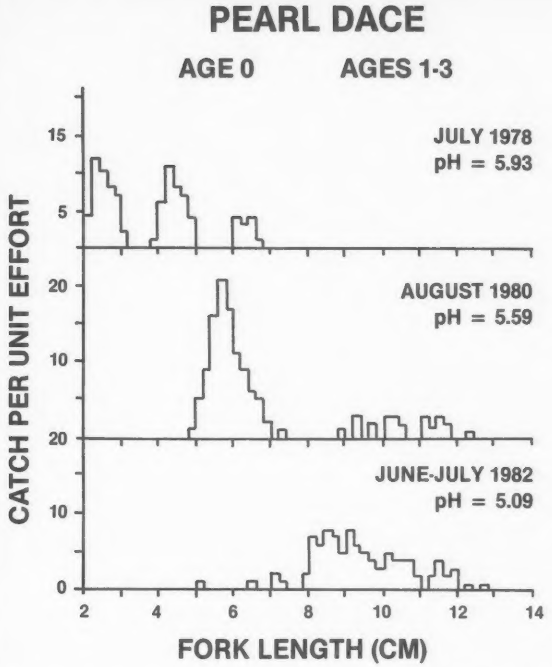

FIG. 11. Catch of pearl dace in 10 24-h trapnet sets for 1978, 1980, and 1982.

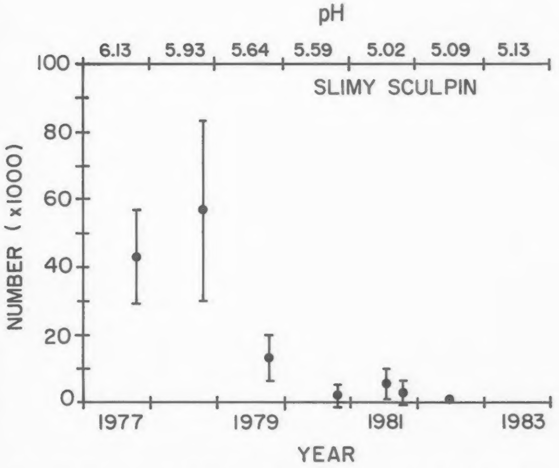

FIG. 12. Estimates and their 95% confidence intervals of the abundance of slimy sculpin in Lake 223 during 1977 to 1983. Too few fish were seen in 1982 to form a confidence interval.

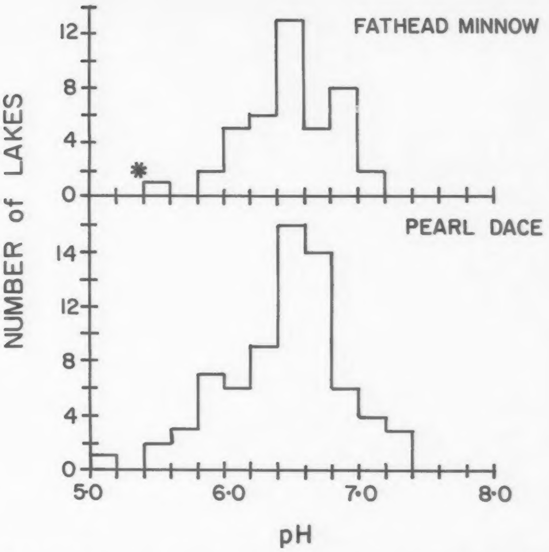

FIG. 13. Presence of fathead minnow and pearl dace in lakes of different pH in the Experimental Lakes Area in 1973 (data from Beamish et al. 1976). * = experimentally acidified Lake 304 fathead minnow have disappeared from this lake since sampling occurred in 1973.

for lake trout abundance decline in Lake 223 was recruitment failure. If lake trout recruitment had continued in 1982 and 1983 at a rate similar to the average 1977 to 1981 rate ($\simeq$167 fish$\cdot$yr^{-1}) and annual survival had decreased as observed (Fig. 3), $\simeq$733 trout (a loss of 121 fish) would have been present in Lake 223 at fall 1983. The actual decrease was from $\simeq$854 to $\simeq$488 fish (a loss of 366 fish). Therefore, increased mortality of Lake 223 trout was responsible for $\simeq$33% of the observed abundance decline. Recruitment failures accounted for most ($\simeq$67%) of the abundance decline. If recruitment failures continue in Lake 223 and annual survival does not decline further, the lake trout population would be reduced to less than 50 individuals in 6 yr.

The threshold for white sucker recruitment failure in Lake 223 was 5.02 (1981, Fig. 9), although recruitment failures occurred in 1982 and 1983 at slightly higher pH (5.09 and 5.13, respectively). This threshold is similar to thresholds inferred from lake surveys of white sucker presence and lake pH in New York Adirondack lakes (pH 5.2, Schofield 1982), and Ontario La Cloche Mountain lakes (pH 4.7–5.2, Beamish 1976; Harvey 1982). It also is very similar to the pH (5.02) where Trojnar (1977) observed increased larval mortality in a laboratory study. Recruitment failures were the primary cause of the white sucker abundance decline in Lake 223 from 1981 to 1983 (Fig. 8). Survival of fish $\geqslant$ age 1 increased during this same time interval. If recruitment failures continue and annual survival remains at the average rate from 1976 to 1983 ($\simeq$0.61 $\cdot$yr^{-1}), the white sucker population would be reduced to less than 50 individuals in 9 yr.

The apparent thresholds for recruitment failure of fathead minnow and pearl dace were 5.93 and 5.09, respectively. We believe that these species spawned at these pH's because gravid females and ripe males were captured and eggs could be extruded from females. We have no length-frequency data for age 0 fathead minnows, but young-of-the-year were observed in 1977 along a sand beach and rocky shore zones in Lake 223. None were observed during succeeding years. Age 0 pearl dace were numerous from 1978 to 1981, very rare in 1982 (Fig. 11), and absent during 1983.

The pH threshold (5.93) for fathead minnow recruitment failures in Lake 223 is similar to the thresholds inferred from surveys of ELA lakes (pH 5.8, Fig. 13) and Algoma–Sudbury

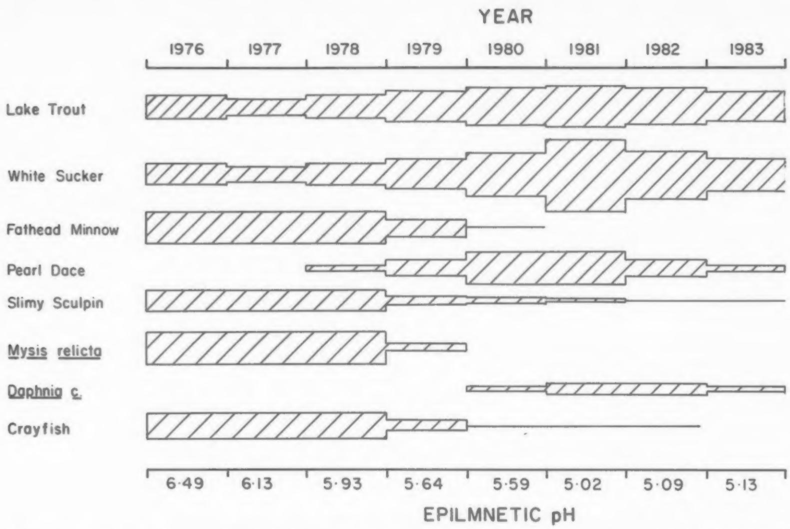

FIG. 14. Relative changes in abundance of lake trout and their food organisms in Lake 223 from 1976 to 1983. Sizes of barred areas are proportional to abundances. Sources of data are: Nero and Schindler 1983 (*Mysis relicta*), I. J. Davies, Freshwater Institute, unpublished data (crayfish *Orconectes virilis*), Malley et al. 1982 and unpublished data (*Daphnia catawba*); and this study (all fish species).

lakes in Ontario (pH 5.9, D. K. McNicol, B. E. Bendell, and R. K. Ross, Canadian Wildlife Service, Ottawa, unpublished data). The pH threshold (5.09) for pearl dace recruitment failures in Lake 223 is slightly lower than the threshold inferred from other ELA lakes (pH 5.4, Fig. 13), from the Ontario Ministry of Natural Resources comprehensive lake survey dataset (pH 5.6, G. L. Beggs, Ontario Ministry of Natural Resources, Toronto, personal communication), and from surveys of Algoma–Sudbury lakes in Ontario (pH 5.7, D. K. McNicol, B. E. Bendell, and R. K. Ross, Canadian Wildlife Service, Ottawa, unpublished data). This may be due to an artifact of our acidification schedule. Pearl dace spawn during the snowmelt and ice-off period in the ELA lakes; this is before yearly additions of acid to Lake 223. Snowmelt water at the ELA is well buffered, and the pH of Lake 223 is usually higher during this time than the remainder of the ice-free season. The relative increase in lake pH immediately after snowmelt was higher in 1981 (pH 5.90) than 1982 (pH 5.41) or 1983 (pH 5.41) (Linsey and Braund 1984; G. A. Linsey, ELA staff, unpublished data). This may explain why the Lake 223 threshold pH for pearl dace recruitment failure is lower than other survey data. This also may explain why the first recruitment failure for pearl dace occurred in 1982 (pH 5.09), although recruitment occurred when the average lake pH was lower in 1981 (pH 5.02).

There is good agreement between the threshold for recruitment failures of Lake 223 fathead minnow and both laboratory and experimental stream studies. Mount (1973) found reduced hatchability of fathead minnow eggs at pH 5.9 in laboratory bioassays. Zischke et al. (1983) found reduced survival of larval fathead minnow in experimental streams at pH 6.0. At this time, there are no comparable laboratory or experimental stream studies of pearl dace.

While we do not have length-frequency data for slimy sculpin, SCUBA divers observed fewer small sculpins from 1979 to 1981 than 1977 to 1978. Age 0 slimy sculpins are generally less than 3 cm in length (Mohr 1984), and it is difficult to determine whether the small sculpins absent from Lake 223 in 1979 were age 0 fish or both age 0 and 1 fish. Therefore, while a series of recruitment failures probably occurred, we do not know whether the threshold pH was 5.93 (1978) or 5.64 (1979). We are not aware of any comprehensive survey data for presence of slimy sculpin in lakes related to pH, but a survey of mottled sculpin (*Cottus bairdi*) in naturally acidic Wisconsin lakes showed this species did not occur in lakes with pH ≤5.6 (Rahel and Magnuson 1983). In addition, in spite of the wide zoogeographic distribution of sculpins over much of Canada's acid sensitive areas, they have received little attention in synoptic surveys.

There is the possibility that all the recruitment failures we observed in Lake 223 were not related to lake pH, but occurred regularly for these fish populations. This is extremely unlikely for at least two reasons. First, we have no evidence of previous recruitment failures in Lake 223 for either lake trout or white sucker. Based on age-frequency distributions, successful recruitment occurred each year for at least 13 yr prior to lake acidification for lake trout and for at least 10 yr for white sucker. The occurrence of four successive recruitment failures for lake trout and three successive recruitment failures for white sucker due to natural variation is highly unlikely. Second, for short-lived species such as pearl dace and fathead minnow, successive recruitment failures would lead to rapid extinction in lakes. This is particularly true for fathead minnow where one or two year-classes form the majority of spawners. To sustain lake populations, successful annual recruitment is a necessity. We found no evidence of extreme abundance fluctuations of cyprinids in reference Lakes 114, 303, and 302. We have also resurveyed many of the lakes Beamish et al. (1976) surveyed in 1973; we have not found any natural extinctions of cyprinids. This is also true for the Beamish et al. (1976) slimy sculpin survey data. We conclude that the recruitment failures for all Lake 223 fish species were due to acidification.

Interactions

When the Lake 223 acidification experiment was initiated, we had hypothesized that many changes would occur for the resident fish populations. Based on scientific literature, we had hypothesized that growth would decrease, abundances would decline, mortality of adults would increase, egg resorption would increase, skeletal deformities would occur, and recruitment failures would occur. Some of these changes occurred, and others did not. We had not anticipated many of the changes that occurred at relatively high pH. In retrospect, changes in the community structure of the Lake 223 fish populations and in lower trophic levels may help explain why some of the expected changes did not occur, or first occurred rather late in the experiment.

We had not anticipated the large number of changes in fish populations and invertebrates (summarized in Schindler et al. 1985) that occurred in 1979 (Fig. 14). We had hypothesized that the abundance declines of *Mysis relicta*, fathead minnow, and crayfish (*Orconectes virilis*) would result in reduced lake trout growth. Instead, lake trout growth (Fig. 1) did not decrease. In addition, lake trout abundance (Fig. 3) increased; we anticipated that this might cause growth decline. Losses of some lake trout prey items were apparently countered by increases in others, such as pearl dace and small white sucker (Fig. 14).

We had not anticipated the very large increase in white sucker abundance (Fig. 8) in 1979. We suspected that this increase might result in decreases of white sucker growth and condition, but growth (Fig. 6) and condition (Fig. 7) did not decline. White sucker growth increased in 1979 and 1980. Chironomids are the primary prey of white sucker in the ELA (ELA staff, unpublished data), and dipteran emergence gradually increased from 1976 to 1981 (Schindler et al. 1985). The combination of increased prey and enhanced habitat for white sucker fry, due to the appearance of extensive *Mougeotia* algal mats in the littoral zone (Schindler 1980), may explain the increased white sucker abundance and growth.

While abundance declines have been reported often for many fish populations as lakes are acidified (Muniz and Leivestad 1980; Haines 1981; Harvey 1982; and others), abundance increases during acidification are not as well known. The increase in lake trout (Fig. 3), white sucker (Fig. 8), and pearl dace (Fig. 11) abundances were not anticipated. The increase in pearl dace abundance was the most surprising of these three increases. Pearl dace abundance was relatively stable in Lake 114 from 1978 to 1983, and other less frequently studied ELA lakes. In Lake 114, the diets of fathead minnow and pearl dace were very similar (Tallman et al. 1984). The disappearance of fathead minnow from Lake 223 may be related to the increase in pearl dace abundance, but these two species often occur together at high abundance levels in other ELA lakes (Beamish et al. 1976; ELA staff, unpublished data).

One of the most perplexing changes in the Lake 223 fish populations during the acidification was the gradual decline in lake trout condition factor from 1972 to 1981 (Fig. 2) . We had hypothesized that growth would slow as condition declined but growth did not decline (Fig. 1) until almost all lake trout prey had disappeared in 1982 (Fig. 14). At this time condition factors declined to a level lower than other ELA populations, and mortality increased. It would be tempting to conclude that a gradual decrease in lake trout condition was an indicator of increasing stress, but at the same time as condition was decreasing from 1977 to 1979, lake trout abundance was increasing (this is discussed further in Mills 1984). An inverse relationship between condition factor and fish abundance is well known in fish research (Nikolsky 1963). In addition, condition factors from 1977 to 1979 were still above levels at the start of acidification.

We had not anticipated the extremely rapid abundance declines of fathead minnow, pearl dace, and slimy sculpin (Fig. 10–12). Laboratory studies (Mount 1973) and experimental streams studies (Zischke et al. 1983) indicated the fathead minnow abundance would decline more gradually. Conversely, we expected lake trout and white sucker abundance to decrease more rapidly due to earlier increased mortality of lake trout adults and general increased mortality of adult white suckers (as described for the George Lake, Ontario, population by Beamish 1974). The increased survival of Lake 223 white sucker in 1982 and 1983 (Fig. 8) may be due to reduced lake trout predation; the small individuals that Lake 223 trout normally preyed on were absent. The rate of abundance decline for all Lake 223 fish species was inversely related to the number of age-groups in each population. Abundance of longer-lived species, lake trout and white sucker, declined much less rapidly than shorter-lived species, fathead minnow and pearl dace. In addition, time-consuming aging of fishes to detect year-class failures of cyprinids was not necessary. This has obvious implications for regional monitoring programs of acid rain impacts based on fish population data. On the basis of our Lake 223 results and survey results, one would expect more rapid, more detectable changes in species with short life-spans, like fathead minnow, than species with relatively long life-spans, like lake trout.

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Histopathological Changes in the Gills of Pearl Dace, *Semotilus margarita,* and Fathead Minnows, *Pimephales promleas,* from Experimentally Acidified Canadian Lakes

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Leino, R. L., P. Wilkinson, and J. G. Anderson. 1987. Histopathological changes in the gills of pearl dace, *Semotilus margarita*, and fathead minnows, *Pimephales promleas*, from experimentally acidified Canadian lakes. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 126–134.

The gills of pearl dace form experimentally acidified Canadian ELA lakes 223 (pH 5.2) and 114 (pH 5.8) and fathead minnows from Lake 114 were examined microscopically and compared with gills from unacidified waters. The acid-exposed gills exhibited (1) hyperplastic primary lamellar epithelia, (2) diminished respiratory lamellar surface areas, (3) widened respiratory blood-water barriers, and (4) altered cellular composition and morphology, especially greater numbers of chloride (ionoregulatory) cells with and without apical pits. While little can be said about the effects of acid water on fish based on such a small sample of lakes, the present research does not contradict the views expressed by many investigators that very soft water with a low pH and toxic metal ions has a deleterious effect on gills and the physiological processes associated with them.

Les branchies de mulets perlés provenant des lacs 223 (pH 5,2) et 114 (pH 5,8) expérimentalement acidifiés de la zone des lacs expérimentaux, de même que celles de ménés tête-de-boule du lac 114, ont fait l'objet d'un examen microscopique et d'une comparaison avec des branchies de poissons provenant d'eaux non acidifiées. Les branchies exposées au milieu acide présentaient : (1) une hyperplasie de l'épithélium primaire lamellaire; (2) une diminution de la surface respiratoire lamellaire; (3) un élargissement des barrières respiratoires sang-eau et (4) une altération de la composition et de la morphologie cellulaire, notamment une augmentation du nombre des cellules à chlorure (ionorégulation) avec ou sans fosses apicales. Le nombre de lacs échantillonnés est insuffisant pour permettre de tirer des conclusions quant aux effets de l'eau acidifiée sur les poissons, mais les résultats obtenus ne contredisent pas les vues exprimées par bon nombre de chercheurs, à savoir que les eaux très douces à faible pH contenant des ions métalliques toxiques ont un effet nuisible sur les branchies et les processus physiologiques qui leur sont associés.

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The Canadian Department of Fisheries and Oceans has been studying the effects of acidification on aquatic ecosystems by adding acid to certain softwater lakes in N.W. Ontario's Experimental Lakes Area. For example, in 1976, after two years of preacidification studies, sulfuric acid was added to one of these lakes (Lake 223) in an effort to decrease the pH by about 0.25 pH units per year (Schindler and Turner 1981); the mean pH of the epilimnion was 5.1 in 1982 compared with an initial pH of 6.5 in 1976. By 1982, several biological effects had been noted including the virtual disappearance of fathead minnows, *Pimephales promelas*, in 1979 (pH 5.6) and a dramatic decline in the population of pearl dace, *Semotilus margarita*, between 1981 and 1982 (Mills et al. 1987).

Numerous factors may contribute to the disappearance of fish populations from acidified lakes (Leivestad 1982). Laboratory studies implicate disturbances in ionoregulatory processes in the gills as the first of several direct physiological effects of acidification on fish (reviewed by McDonald 1983). Histological evidence for a physiological effect on ionoregulatory mechanisms is provided by laboratory studies showing that chloride (ionoregulatory) cells in gills of fathead minnows react to chronic acid stress by proliferation and by changes in their morphology (Leino et al. 1983; Leino and McCormick 1984). The purpose of the present study was to determine (1) if the same histological changes which occur in the gills of acid-stressed, laboratory-exposed fish are also seen in fish from two experimentally acidified Canadian lakes, and (2) if different histological or histopathological changes occur in gills of fish from these acidified lakes.

Materials and Methods

Sources of Fish

Pearl dace, the chief species examined in the present study, were mostly obtained from samples trap netted on 20 May and 1 June 1981 from Lakes 223 (acidified, $n = 13$ fish) and 302[1] (control, $n = 11$ fish). Preliminary water chemistry data for the ELA lakes were provided by the ELA staff (unpublished data). Mean epilimnetic pH's of Lake 223 were: '74, 6.70; '75, 6.64; '76 — acid added — 6.49; '77, 6.13; '78, 5.93; '79, 5.64; '80, 5.59; '81, 5.16; '82, 5.11. The pH of Lake 302 remained close to 6.5. Other chemical characteristics of Lakes 223 and 302 and the other lakes referred to in the present study

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[1] Lake 302 was subsequently used as an experimental lake for acid additions.

TABLE 1. Some chemical characteristics of the lakes referred to in the present study.

Lake	Approximate mean pH	Alkalinity (μeq/L)	Ions (mg/L)				
			Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
Unacidified							
Lake 302[a]	6.5	57	0.85	0.41	1.71	0.62	0.47
Trilby Lake[b]	6.5	<50	—	—	—	—	—
Experimentally acidified							
Lake 223[a]	5.16	−2	0.46	1.07	0.34	2.77	0.63
Lake 114[a]	5.95('81)	29	0.67	0.82	1.01	1.75	0.73
	5.8 ('83)	—	—	—	—	—	—
Lake Superior[c] (laboratory exposures)	5.5	110	1.1	1.2	0.4	13.6	3.1

[a] Preliminary data provided by the ELA staff.

[b] Estimates based on Omernik and Griffith (1985) and personal communication with S. Serns, Wisconsin Dep. of Natural Resources.

[c] Leino and McCormick (1984).

TABLE 2. Mean epilimnetic concentrations (in μg/L) of certain metal ions in Lake 223 (source: preliminary data provided by ELA staff).

Ion	1976 (pH 6.5)	1981 (pH 5.2)	1982 (pH 5.1)
Al	10.2	35.8	40.8
Cu	1.6	1.7	5.2
Fe	15.7	35.0	59.4
Mn	5.5	174	154
Zn	1.8	6.5	8.1

are listed in Table 1. Mean epilimnetic concentrations of several potentially toxic metal ions (in Lake 223; Table 2), while very low, rose during the study period.

In addition to pearl dace from Lakes 223 and 302, a preliminary examination was made of the gills of dace netted in May, 1981 (n = 12) and in April and May, 1983 (n = 17) and fathead minnows netted in May, 1983 (n = 11) from ELA Lake 114. This lake was experimentally acidified to an approximate pH of 5.95 in 1981 and 5.8 in 1983. Gills of fathead minnows were examined because this species was used in laboratory studies on the effects of chronic acid exposures using water from Lake Superior (Leino and McCormick 1984). Gills from 12 fathead minnows, netted in August, 1981, from an unacidified softwater lake in Vilas County, WI, USA. (Trilby Lake) were also examined since none were obtained from the Canadian control lakes.

Tissue Preparation

Fish were fixed in 10% neutral buffered formalin at the lake sites and subsequently transferred to the University of Minnesota, Duluth campus. The first gill arch from each gill chamber was then processed using standard methods for embedding in Dupont JB-4 methacrylate (Polysciences).

Morphometry

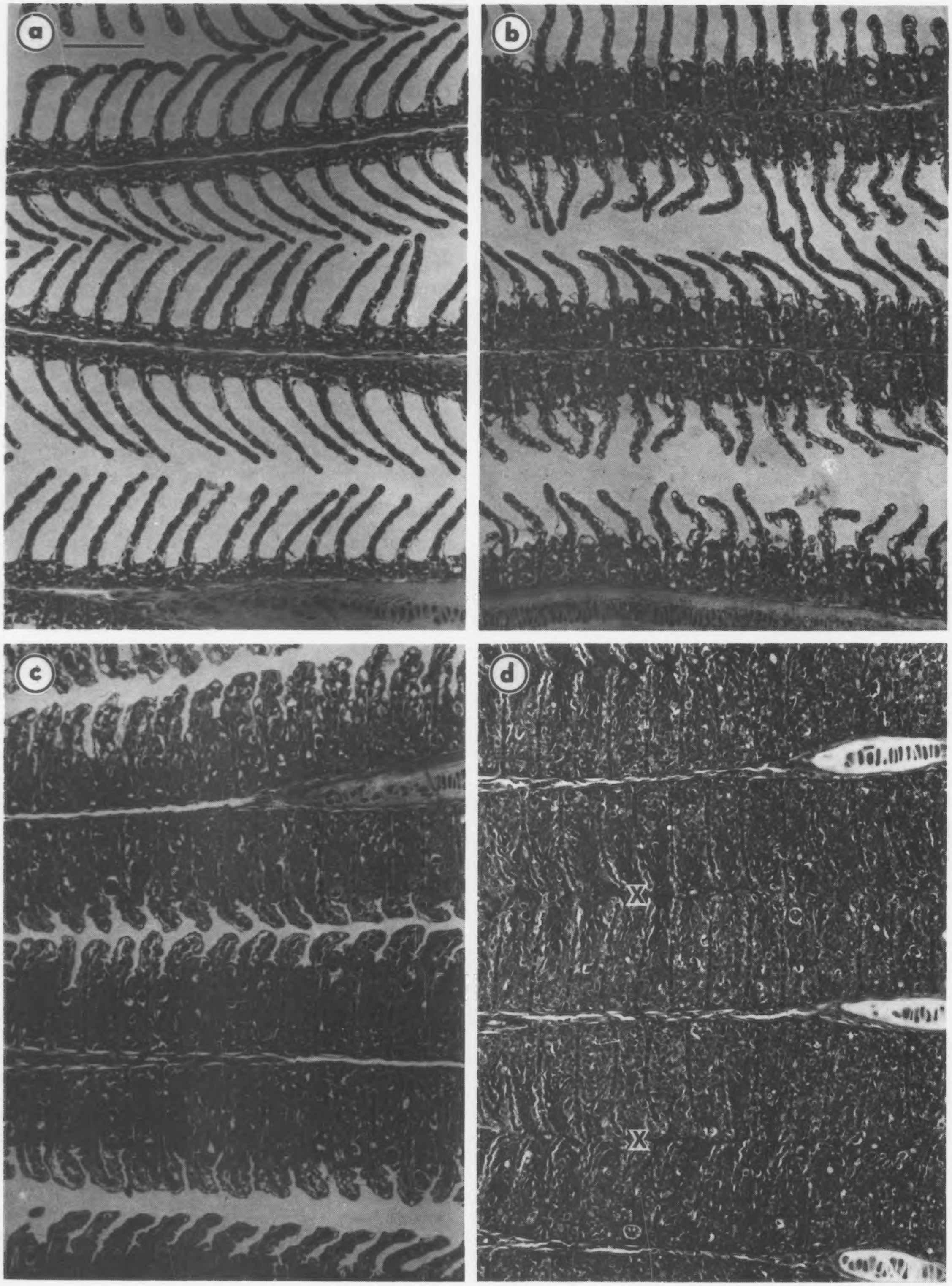
Morphometric studies were done on gills of dace from Lakes 223 and 302. Gills were oriented and sectioned as described by Leino and McCormick (1984): (1) the medial hemibranchs were placed downward in embedding molds; (2) longitudinal sections for cell counts were made with a Sorvall JB-4 microtome set at 2 μm from comparable areas on each medial hemibranch, i.e., from an approximately 200 μm long portion of a filament sectioned through the region between the efferent artery and the gill ray. In healthy dace, this region has a primary lamellar epithelium of relatively uniform thickness and cell composition with a good representation of the various gill cell-types and also includes the central part of the respiratory (secondary) lamellae; (3) sections were stained in eosin followed by Harris' hematoxylin; (4) slides were randomized and coded by a colleague prior to examination; (5) chloride, mucous, granular and rodlet cells in the epithelium of both primary and secondary lamellae were counted.

The width of the primary lamellar epithelium (measured from the basement membrane to the epithelial surface) and the length of the respiratory lamellae (i.e., beginning from the level of the primary lamellar epithelial surface) were measured at 1000× with an eyepiece micrometer using the gill sections previously described. Eighteen measurements of the thickness of the epithelium of the primary lamellae were made for each fish as follows: two measurements each in the inner, middle and outer one-third of three different gill filaments. the location of each measurement within a designated region was selected using a random number table. Respiratory lamellar length was measured using a similar procedure.

The thickness of the blood−water barrier was determined according to Hughes and Perry (1976), utilizing a Merz grid and Zeiss projection microscope at 2000×. The Merz grid permits randomly selected measurements of a regularly arranged structure such as the gill; 20 measurements of the distance between the external surface of the respiratory epithelium and the nearest blood space were taken for each fish. At least two separate regions of gill were sampled.

Statistical Analyses

Most of the data from Lakes 223 and 302 had greatly dissimilar distributions and variances so a non-parametric statistic, the Mann-Whitney U test, was used to evaluate differences between samples (Hull and Nie 1981). Some results were predicted on the basis of previous experiments (Leino and McCormick 1984). In these instances, one-tailed tests were used.


a
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Results

Microscopic Appearance of the Gills

The microscopic structure of the gills of dace from Lakes 223 and 302 showed marked differences (Fig. 1–2). Gills from Lake 223 exhibited hyperplasia, a wider blood–water barrier, and a difference in the morphometrics and morphology of their chloride cells.

Hyperplasia

A moderate to severe hyperplasia of the epithelium of the primary lamellae occurred in all gills from the Lake 223 sample (Fig. 1b–d, 2b). By partially filling in the spaces between adjacent respiratory lamellae, the hyperplastic epithelium contributed to a reduction in the mean length of the respiratory lamellae and therefore to a decrease in respiratory surface area (Table 3). The primary lamellar epithelium in Lake 223 dace averaged 71% thicker and the respiratory lamellae were 36% shorter than in control dace. The hyperplasia in about 10% of the filaments of Lake 223 gills was so extensive that it obliterated the spaces between adjacent respiratory lamellae. In some instances the epithelia of adjacent *primary* lamellae were fused making part of the gill a "solid" structure with no respiratory surfaces (Fig. 1d). (No blood–water barrier measurements or measurements involving lamellar hyperplasia were made in these regions.).

Hyperplasia of the primary lamellar epithelium was also observed in gills of dace and fathead minnows from Lake 114. Seven of 12 dace in the 1981 sample (lake pH 5.95) exhibited hyperplastic epithelium like that shown in Fig. 1b. All of the gills from the dace and fathead minnows (Fig. 4c) in the 1983 sample (pH 5.8) had varying degrees of hyperplasia, mostly similar to that shown in Fig. 1b and 1c. On the other hand, the gills of fathead minnows exposed to acid in the laboratory or from Trilby Lake had no hyperplasia (Figs. 4a, b) and looked similar to the gills of Lake 302 dace (Fig. 1a).

Respiratory Barrier

The width of the blood–water barrier of the respiratory lamellae was significantly greater in gills of dace from Lake 223 (Table 4). The increase in diffusion distance involved two factors: (1) epithelial cell hypertrophy, that is, many epithelial cells appeared to be enlarged or swollen (Fig. 3b) making the "mean width of blood–water barrier through regions without lymph spaces" value in Table 4 greater, and (2) an increase in the extent of microscopically visible "lymph spaces," fluid filled spaces between the two layers of cells comprising the respiratory epithelium (Fig. 3a; also see Temmink et al. 1983) which widen the blood–water barrier. Dace and fathead minnows from Lake 114 showed similar respiratory barrier changes.

TABLE 3. Primary lamellar hyperplasia: differences in width of primary lamellar epithelium and length of secondary lamellae in Lake 223 ($n = 13$) and Lake 302 ($n = 11$) pearl dace.

Measurement in μm	Lake 223 (acidified pH 5.16)	Lake 302 (non-acidified pH 6.5)
Mean width of primary lamellar epithelium[a] (SD)	60 (25)	35 (9)
Minima–maxima, primary lamellar widths	22–153	15–70
Mean length of respiratory lamellae[a]	72 (29)	113 (21)
Minima–maxima, respiratory lamellae lengths	0–150	46–180
Mean TL of fish in mm	84 (12)	93 (7)

[a] Significance of difference between lakes: $P < 0.0001$, Mann-Whitney U two-tailed test.

TABLE 4. Differences in respiratory barrier measurements between gills of Lake 223 ($n = 13$) and Lake 302 ($n = 11$) pearl dace.

Measurement in μm	Lake 223	Lake 302
Mean width of blood–water barrier[a] (SD)	3.5 (2.2)	2.4 (2.4)
Minima–maxima, blood–water barrier widths	0.5–13.0	0.3–13.7
Mean width of blood–water barrier through regions without lymph spaces[b]	2.7 (1.5)	1.5 (1.0)
Mean width of blood–water barrier through regions with lymph spaces	4.6 (2.4)	4.5 (3.3)
Mean percentage of total measurements through regions with lymph spaces[c]	43.5%	29.5%

[a] Significance of difference between lakes: $P < 0.0001$, Mann-Whitney U two-tailed test.

[b] Significance of difference between lakes: $P < 0.001$, Mann-Whitney U two-tailed test.

[c] Significance of difference between lakes: $P < 0.05$, Mann-Whitney U two-tailed test.

Cytological Differences

Five of the best-stained gills from Lake 223 and 302 dace, respectively, were selected for detailed cytological study. In this subsample, gills of dace from Lake 223 had much higher mean numbers of chloride cells (Table 5), many of which had apical pits (Fig. 3b) or evaginations similar to those of fathead minnows exposed to acid in the laboratory (Leino and McCormick 1984). Pits and evaginations were rare or absent in chloride cells of Lake 302 dace. In addition, the percentage of chloride cells lying exclusively in the epithelium of the secondary lamellae was also much greater in the gills of Lake 223 dace (Table 5) as was the case with fathead minnows exposed

FIG. 1. Section of gill of pearl dace (a) from control Lake 302 showing normal gill structure; (b–d) from acidified Lake 223 (pH 5.16) showing various degrees of hyperplasia. In "c" the respiratory lamellae are short and thickened. In "d" hyperplasia has obliterated the respiratory lamellae and adjacent primary lamellae are fused (X). Magnifications, ×160; scale line = 100 μm.

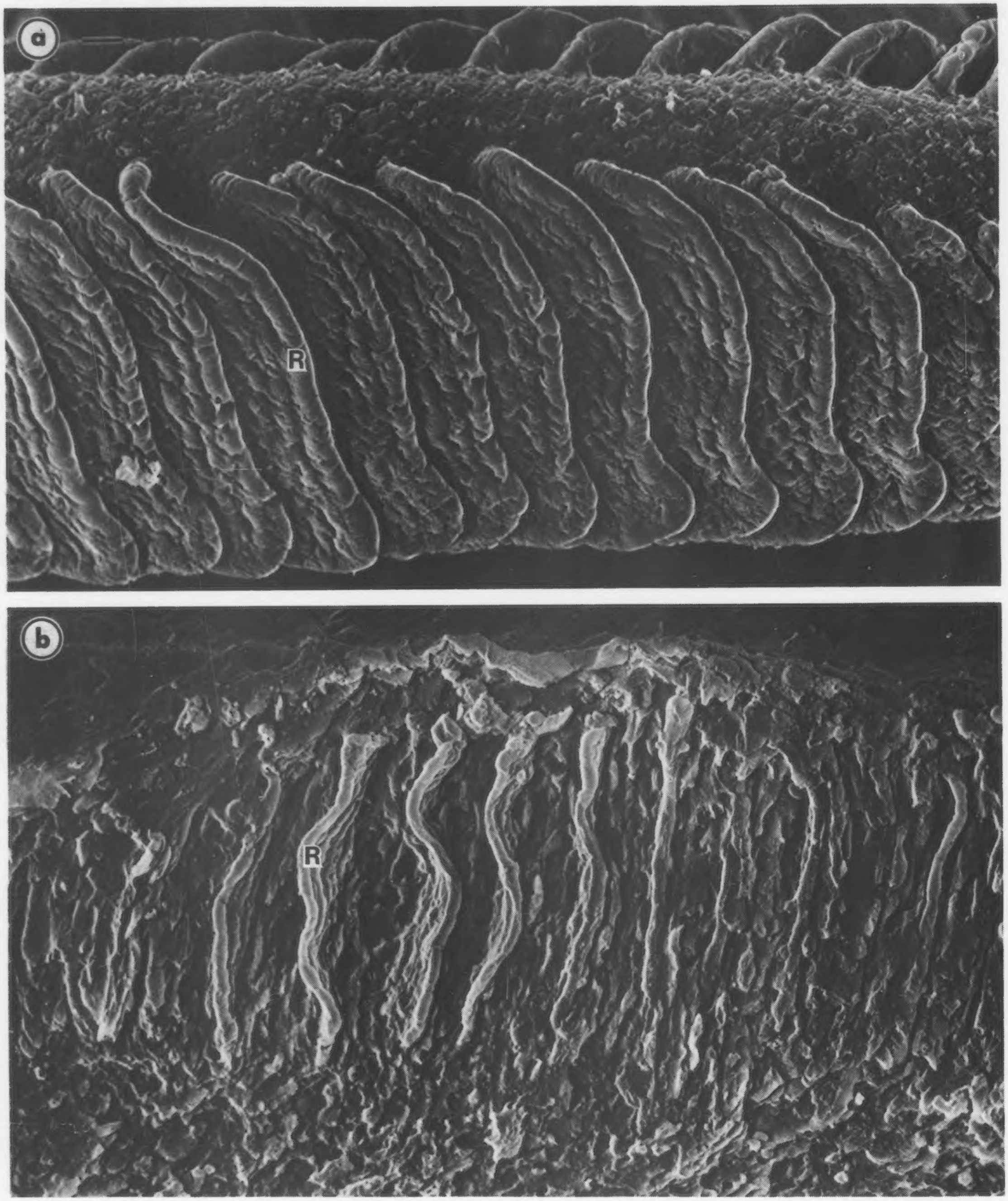

FIG. 2. (a) Scanning electron micrograph of gill of dace from control Lake 302. Respiratory lamellae (R) project from primary lamella. ×690; scale line = 10 μm; (b) Scanning electron micrograph of gill of dace from acidified Lake 223. Respiratory lamellae are shortened or obliterated by hyperplasia of primary lamellar epithelium. ×690.

to acid in the laboratory (Leino and McCormick 1984).

Besides chloride cells, mucous, rodlet, and granular cells were counted. While the numbers of mucous cells tended to be greater in Lake 223 gills, especially minimum numbers, the differences were not statistically significant (Table 6). In general, the hyperplastic interlamellar epithelium of acid-exposed dace contained more pavement epithelial cells and fewer rodlet and granular cells than normal epithelium (Table 6).

TABLE 5. Differences in chloride cell morphology and morphometry between gills of Lake 223 (n = 5) and Lake 302 (n = 5) pearl dace.

Measurement	Lake 223	Lake 302
Mean total numbers of chloride cells per cm[a] (SD)	1297 (563)	406 (203)
Minima–maxima mean total numbers of chloride cells per cm	656–2200	122–648
Mean percentage of chloride cells with apical pits	17.7%	0.37%
Minima–maxima, percentage of chloride cells with apical pits	5.1–27.3%	0–1.6%
Mean percentage of chloride cells with apical evaginations	1.1%	0.0%
Mean percentage of chloride cells in the secondary lamellar epithelium	34.6%	6.2%
Minima–maxima, percentage of chloride cells in the secondary lamellar epithelium	13.4–44.4%	3.7–11.5%

[a] Significance of difference between lakes for all means: $P < 0.004$, Mann-Whitney U one-tailed test.

TABLE 6. Counts of mucous, granular, and rodlet cells in gills of Lake 223 (n = 5) and Lake 302 (n = 5) pearl dace.

Estimated number of cells per cm[a]	Lake 223	Lake 302
Mucous cells (SD)	510 (60)	371 (192)
Minima–maxima, mucous cells	450–600	127–638
Granular cells	32 (30)	109 (55)
Rodlet cells	6 (7)	34 (36)

[a] Difference between lakes significant only for granular cells: $P < 0.02$, Mann-Whitney U two-tailed test.

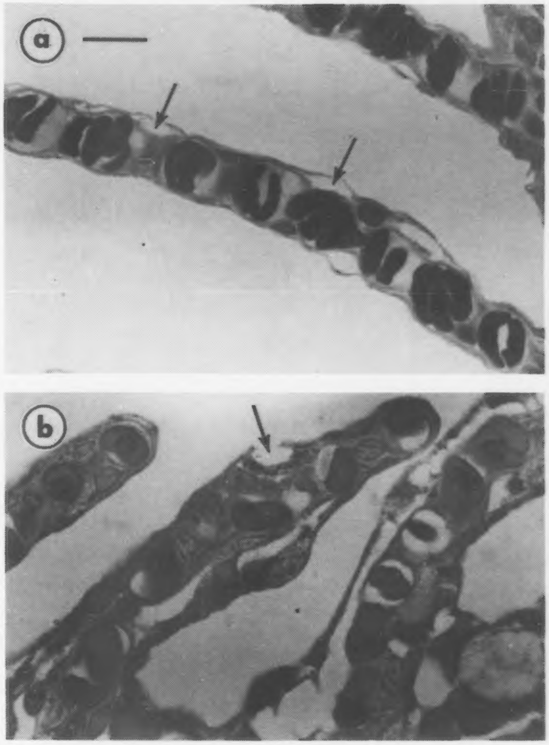

FIG. 3. Section through respiratory lamellae in Lake 223 dace gill (a) showing lymph spaces (arrows). × 1045; scale line = 10 μm. (b) showing chloride cell with apical pit (arrow). × 1030.

Discussion

After 6 years of acidification of Lake 223, the population of pearl dace declined dramatically (Mills et al. 1987). In the present study, gills of pearl dace obtained in the spring of 1981 were examined for histological and histopathological changes, assuming that if gills are the primary "target organ" of acidification (reviewed by Leivestad 1982; McDonald 1983), then they might exhibit a significant histological reaction to chronic acid stress. Indeed, gills of pearl dace from Lake 223 acidified to pH 5.16 exhibited: (1) a hyperplasia of the epithlium of the primary lamellae, (2) a reduction of the surface area of the secondary lamellae, (3) an increase in the thickness of respiratory blood–water barrier, and (4) an alteration in cellular composition and morphology. The first three of these changes would probably reduce ion losses through the gills (ion losses are a common result of acidification, e.g., see Packer and Dunson 1970, 1972; Leivestad and Muniz 1976; McWilliams and Potts 1978; Neville 1979; McDonald et al. 1980; McDonald and Wood 1981; Booth et al. 1982; Lee et al. 1983; Saunders et al. 1983; Fraser and Harvey 1984; Giles et al. 1984; Hobe et al. 1984) but would also reduce respiratory capacity and efficiency. Leino and McCormick (1984) have suggested that the fourth change, in particular the increased numbers of chloride cells and alterations in their morphology, is associated with stressed ionic or acid-base balancing systems.

Comparison with Laboratory Studies

Chloride cells of Lake 223 pearl dace showed the same sorts of numerical and morphological changes as described in fathead minnows exposed to a similar pH (5.0) for 129 days in the laboratory (Leino et al. 1983; Leino and McCormick 1984). Leino and McCormick (1984) suggested that the greater number of chloride cells with increased surface areas in acid-stressed fish may be an adaptation to promote ion exchanges, i.e., NaCl uptake and H_4^+ egress (Na^+/H^+, NH_4^+ exchanges), Ca^{2+} uptake, or even sulfate elimination. The functions of chloride cells in freshwater fish are not well understood but there is evidence (besides an ultrastructure characteristic of ion-transporting cells) that chloride cells take up ions, particularly Na^+, Cl^-, and Ca^{2+} (Bierther 1970; Mattheij and Stroband 1971; Laurent and Dunel 1980; Payen et al. 1981; Gaino et al. 1984; Flik et al. 1984). Notable with regard to the present study are the observations by Mattheij and Stroband (1971) and Laurent and Dunel (1980) showing that when fish

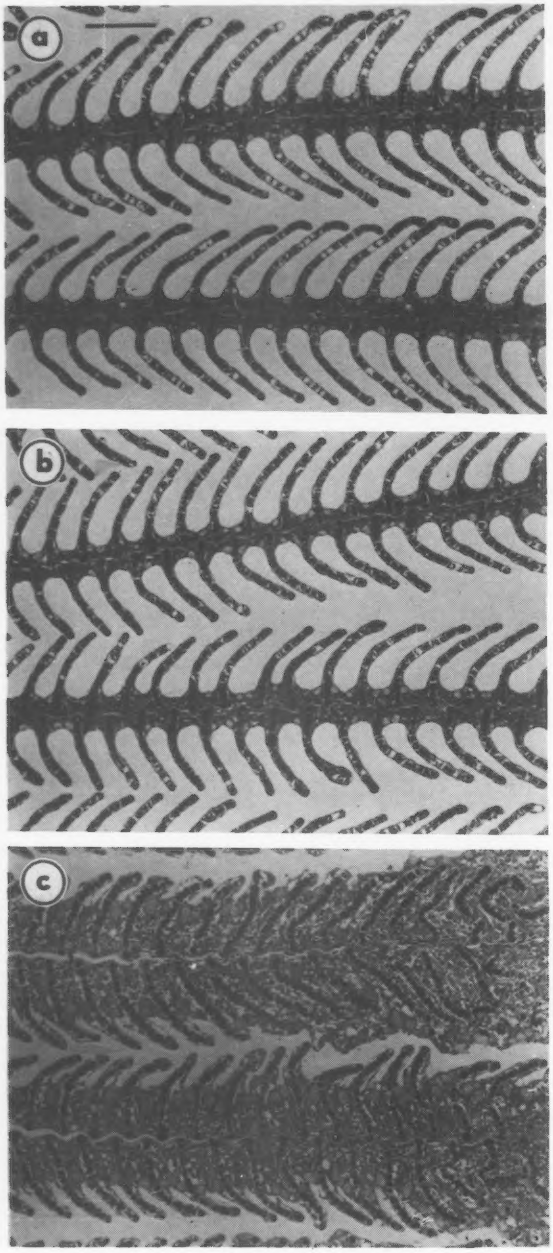

FIG. 4. (a) Section through gill of fathead minnow from unacidified Trilby Lake, WI (pH ~ 6.5), showing normal gill architecture. (b) Section through gill of fathead minnow exposed to Lake Superior water in the laboratory at pH 5.5 for 129 days, showing normal-appearing structure. (c) Section through gill of fathead minnow from experimentally acidified Lake 114 (pH ~ 5.8). These gills exhibit extensive hyperplasia of the epithelium of the primary lamellae with a consequent shortening of the respiratory lamellae. Magnifications, ×115; scale line = 100 μm.

are exposed to deionized water, chloride cell numbers increase dramatically, especially in the secondary lamellar epithelium. These investigators concluded that chloride cell proliferation provides a compensating mechanism for increasing net ion uptake when fish are exposed to conditions which promote abnormally high ion losses. This situation could also exist with fish exposed to acidified water: acid-associated osmotic stress may result in a proliferation of chloride cells.

In the present study, primary lamellar hyperplasia and a wider blood–water barrier were seen only in fish from acidified Lakes 223 and 114. While it is not possible to show, with such a small sample of lakes, whether acid or other factors contributed to the pathology found in these gills, similar pathological changes have recently been reported by Chevalier et al. (1985) in the gills of brook trout from three acidified lakes in Quebec, Canada.

Hyperplasia and blood–water barrier thickening were *not* seen with laboratory exposures of fathead minnows exposed to pH 5.5 for 129 days in Lake Superior water or in fathead minnows from Trilby Lake. Again, the small sample size prohibits drawing definite conclusions, but, in agreement with Chevalier et al. (1985) and others, it is suggested that a combination of stressors, e.g., low pH, low calcium concentrations, increased concentrations of metal ions, might contribute to more severe gill pathology in acidified lakes than, for example, in acidified water with relatively high concentrations of calcium and lack of potentially toxic metal ions as was the case with the laboratory exposed fathead minnows (Table 1; Leino and McCormick 1984).

At normal pHs, hyperplasia or respiratory barrier changes can result from exposure to many gill irritants (Eller 1975) among which are toxic metal ions. For example, Hughes and Perry (1976) reported that rainbow trout exposed to 2 mg/L of Ni^{2+} showed an increase in the thickness of their respiratory blood–water barrier similar to that described in the present study. Higher concentrations of various metal ions may produce more severe gill changes: frank separation of epithelial layers in respiratory lamellae, fusion of respiratory lamellae, epithelial necrosis, chloride cell hyperplasia, or excess secretion of mucus (Skidmore and Tovell 1972; Crespo et al. 1981; Gupta and Rajbanshi 1981; Kumar and Pant 1981; Khangarot 1982; Tuurala and Soivio 1982; Stromberg et al. 1983; Daoust et al. 1984).

In Lakes 114 and 223 the amounts of dissolved Al, Cu, Fe, Mn, and Zn typically rose during acidification but the mean concentrations of these elements were still very low. Concentrations of these ions were often much higher, however, in the 2 or 3 months prior to when the fish samples were taken. Whether long-term exposure to very low or variable concentrations of metal ions can contribute to gill pathology in an acid environment should be explored. As Beamish (1976) has pointed out, most metal toxicity tests are performed at higher pH values, and "safe" levels derived from these experiments may be too high for acidic lakes.

In summary, gills of pearl dace and fathead minnows from experimentally acidified Canadian lakes exhibited various cellular, histological and histopathological changes which may signify or contribute to problems with respiration and ionic and acid-base balances. The population decline of pearl dace and fathead minnows in Lake 223 was primarily the result of recruitment failure (Mills et al. 1987). The ion-imbalancing effects of acid stress on fish could contribute to recruitment failure by reducing reproductive fitness (Beamish 1976; Craig and Baksi 1977; Lee et al. 1983). In addition, if stresses resulting from environmental acidification compromise respiration and ionoregulation in adults, then they could also affect these processes more severely in younger life stages (Robinson et al. 1976; Craig and Baski 1977; Daye and Garside 1980. Rask 1984).

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Early Responses of Periphyton to Experimental Lake Acidification

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We studied the periphyton in two small lakes acidified experimentally with either sulfuric or nitric acid. For comparison, we monitored periphyton in one previously acidified (sulfuric) lake and in two neutral lakes. Net photosynthesis declined in epilithic periphyton in the middle littoral zone (1–2 m) of both recently acidified basins in response to reduced concentrations of dissolved inorganic carbon. Dark respiration and taxonomic composition remained largely unchanged in this zone. Attached filamentous algae proliferated early (≥pH 6.0) in the upper littoral zone (<1 m) of the sulfuric acid basin confirming earlier observations made in the previously acidified lake. These Zygnematales-dominated filamentous algae (largely *Mougeotia* sp.) developed conspicuously on submerged rock and log surfaces. In contrast, growth on macrophytes was variable in both the neutral and nitric acid systems. We hypothesize that during the early stages of acidification proliferation of filamentous Chlorophyceae is due to selection of algae tolerant of increased H^+ and better adapted to obtain inorganic carbon at low concentrations, in combination with reduced grazing.

Nous avons étudié le périphyton dans deux petits lacs acidifiés expérimentalement soit avec de l'acide sulfurique ou de l'acide nitrique. Pour comparer, nous avons contrôlé le périphyton dans un lac déjà acidifié (acide sulfurique) et dans deux lacs neutres. La photosynthèse nette a diminué au niveau du périphyton croissant sur les pierres dans la zone médiolittorale (1–2 m) des deux bassins récemment acidifiés suite à des concentrations réduites de carbone inorganique dissous. La respiration et la composition taxonomique à l'obscurité sont demeurées en grande partie inchangées dans cette zone. Les algues filamenteuses fixées ont proliféré tôt (≥pH 6.0) dans la zone littorale supérieure (<1 m) du bassin contenant de l'acide sulfurique confirmant les observations antérieures faites dans le lac déjà acidifié. Ces algues filamenteuses dominées par des zygnémales (surtout *Mougeotia* sp.) se sont développées visiblement sur des surfaces rocheuses et des rondins submergés. Par contre, la croissance sur les macrophytes a été variable dans les systèmes neutres et contenant de l'acide nitrique. Nous émettons l'hypothèse qu'au cours des premiers stades d'acidification, la prolifération de chlorophycées filamenteuses est attribuable à la sélection d'algues qui tolèrent une augmentation des ions H^+ et qui sont mieux adaptées pour obtenir du carbone inorganique à de faibles concentrations, associée à un broutage réduit.

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While acidification of lakes was harmful to most biota, development of filamentous algae in the littoral zone was enhanced in Scandinavia (Eloranta and Kunnas 1976; Almer et al. 1978; Lazarek 1983) and North America (Hendrey and Vertucci 1980; Stokes 1980). When Lake 223 (Experimental Lakes Area, northwestern Ontario) was acidified experimentally with sulfuric acid, similar aggregations of filamentous Chlorophyceae developed, dominated principally by *Mougeotia* (Schindler et al. 1980c; Müller 1980). The extent and pattern of periphyton development was not quantified in any of the above studies. Moreover, the threshold pH for development of filamentous algae in the littoral zone has not been well established, although *Mougeotia* was abundant in an experimental mesocosm held at a pH of about 6.0 (Müller 1980).

There are several untested hypotheses to explain the increase

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TABLE 1. Morphometric and chemical features of the study lakes (Beaty 1981; Schindler et al. 1980b, c; D.W. Schindler, unpublished data). Water chemistry values are for the epilimnion during the openwater period, and reflect preacid conditions for the acidified lakes.

		Reference lakes		Treated lakes		
				223	302N	302S
Parameter	Units	239	382	(H_2SO_4)	(HNO_3)	(H_2SO_4)
Surface area	ha	54	36	27	13	11
$A_d:A_o$[a]		6.3	4.7	5.0	6.8	3.8
Mean depth	m	10.9	5.9	7.2	5.7	5.1
Years considered		81–84	84	74–75	81	81
H^+	pH	6.9	6.8	6.7	6.6	6.7
Alkalinity	$\mu eq \cdot L^{-1}$	144	88	—	57	60
DIC	$\mu mol \cdot L^{-1}$	150	92	133	78	82
Chl (planktonic)	$\mu g \cdot L^{-1}$	1.9	1.6	1.9	4.1	3.0
Total P	$\mu g \cdot L^{-1}$	8	12	7	12	8

[a] $A_d:A_o$ = ratio of drainage area (A_d) to lake surface area (A_o). A_d of L302N includes A_d and A_o of L302S.

in benthic algal biomass associated with acidification of lakes. Benthic primary productivity may be increased (Almer et al. 1978) due to the development of acidophilic species (Müller 1980). Alternatively, respiration in the community, and hence decomposition, may be reduced with the nonviable component of the periphyton increasing proportionately (Hendrey et al. 1976; Hall et al. 1980). Loss of invertebrate scrapers (Friberg et al. 1980; Hall et al. 1980; Økland 1980) with a concomitant reduction in grazing pressure, as seen in acidified streams, is yet another possibility (Hendrey 1976; Hall et al. 1980; Hendrey et al. 1980).

The purpose of this study was to investigate the responses of periphyton during the early stages (pH > 5.6) of an experimental whole-lake acidification. Specifically, we examined the taxonomy, density, and carbon metabolism of epilithic periphyton in the middle littoral zone. For the metabolism investigation we equate the term epilithic periphyton with that of haptobenthos (Hutchinson 1975), so that our measurements of photosynthesis and respiration represent community metabolism. We also documented the shoreline occurrence, depth distribution, growth thickness and taxonomic characteristics of filamentous algae throughout the littoral zone. For the shoreline mapping of filamentous algae, we describe algae found on rocks and logs as epilithic, and those found on macrophytes as epiphytic. We then examined the hypotheses that as a result of acidification: conspicuous increases in periphyton biovolume in the littoral zone are caused solely by accumulation of viable filamentous green algae; respiration is reduced and photosynthesis is increased in epilithic periphyton in the middle littoral zone.

Study Lakes

Major Morphometric Features

The study lakes are located on the Precambrian Shield of northwestern Ontario (Brunskill and Schindler 1971). All are small and moderately deep (Table 1). Aside from Lake 302N (L302N) which is downstream from L302S, and L223 which is downstream from oligotrophic L224, these lakes are headwater lakes. Their littoral zones contain rock shelves, large boulders, gravel, sand, organic sediment and various aquatic macrophytes (Stockner and Armstrong 1971; Schindler et al. 1973).

Experimental Status of Lakes

In the general experimental design, each recently acidified lake (L302N and L302S) acted as its own control for about 1 yr before treatment. Other lakes (L239 and L382) remained as control or neutral reference lakes throughout the study. A previously acidifed lake (L223) was monitored to verify observations made in the recently acidified lakes.

Beginning in 1976, sulfuric acid was added to L223, during the openwater period each year to lower the epilimnetic pH gradually from 6.7 (Table 1) to 5.1 by 1981 (Cruikshank 1984; Schindler et al. 1985). The epilimnion was maintained at an approximate mean pH of 5.1 during 1982 and 1983, and allowed to rise to 5.4 in 1984.

Although L239 was monitored as a reference system to the acidification experiments, its watershed was perturbed by a wind storm in 1973, and forest fires in 1974 and 1980. Nutrient release from the L239 watershed was elevated temporarily after the windstorm and fires, but this did not greatly affect the nutrient concentration in the lake (Schindler et al. 1980a; Bayley and Schindler 1986), and probably did not have an appreciable effect on its periphyton (Turner 1981).

Prior to 1982, L302S (the southern and upstream basin of double-basin L302) was unperturbed (Schindler et al. 1980b). Exchange of water between L302S and L302N (the downstream basin) was restricted by installing vinyl-impregnated nylon curtains to separate the basins in 1981. Beginning in July, 1982, sulfuric acid was added to the epilimnion of L302S during each openwater period. Additions were made to maintain epilimnetic pH at about 6.2 in 1982, 5.9 in 1983, and 5.6 in 1984 (Fig. 1a).

Prior to acidification, nutrients (C, N, and P) were injected into the hypolimnion of L302N from 1972 to 1976 and in 1978 as part of a waste management experiment (Schindler et al. 1980b). This procedure had relatively little effect upon epilimnetic chemistry and phytoplankton (Schindler et al. 1980b). Beginning in July 1982 nitric acid was added to L302N at the same time as sulfuric acid was added to L302S. The amount added was adjusted for differences in epilimnetic volume and normality of acids (Cruikshank 1984). Because L302N is downstream from L302S it also received indirect additions of H^+ and SO_4^{-2}, increasing epilimnetic sulfate values slightly from 3.0 $mg \cdot L^{-1}$ in 1981 to 4.1 $mg \cdot L^{-1}$ in 1984.

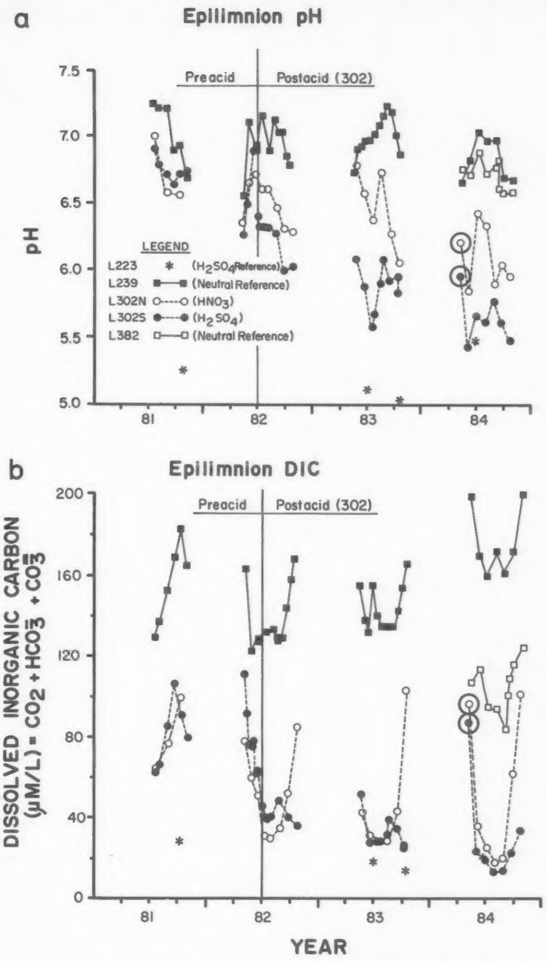

FIG. 1. pH (a) and DIC (b) by epilimnia of the study lakes at times of sampling beginning in July 1981. Acidification of L302N (HNO_3) and L302S (H_2SO_4) was initiated in July 1982, while L223 (H_2SO_4) was acidified beginning in 1976. Circled values represent occasions when sampling occurred prior to the first addition of acid in 1984.

A second reference lake, L382, is more similar to L302N and L302S (prior to acidification) in mean depth and dissolved inorganic carbon (DIC = CO_2 + HCO_3^- + CO_3^{-2}) than L239 (Fig. 1b, Table 1). A southern bay (9% of its surface area) was partitioned in 1978, and although 8% of its watershed was burned in 1979 (Beaty 1984), epilimnetic chemistry remained unchanged (D. W. Schindler, unpublished data).

Epilimnetic Chemistry

Water of ELA oligotrophic lakes (total P ranges from 6 to 12 $\mu g \cdot L^{-1}$) is low in alkalinity (≤ 150 $\mu eq \cdot L^{-1}$) and is therefore acid sensitive. The pH of L223, L302N, and L302S prior to acidification was similar to that of the neutral reference lakes (Table 1).

Chemical changes accompanying the decline of pH in L223 and L302S included increases in total dissolved Mn, SO_4^{-2}, Ca, and conductivity, while nutrients such as P and N remained unchanged (Schindler et al. 1980c; Schindler and Turner 1982; D. W. Schindler, unpublished data). We believe that the most significant chemical change to photosynthetic organisms was the dramatic decline in DIC (Fig. 1b, 2).

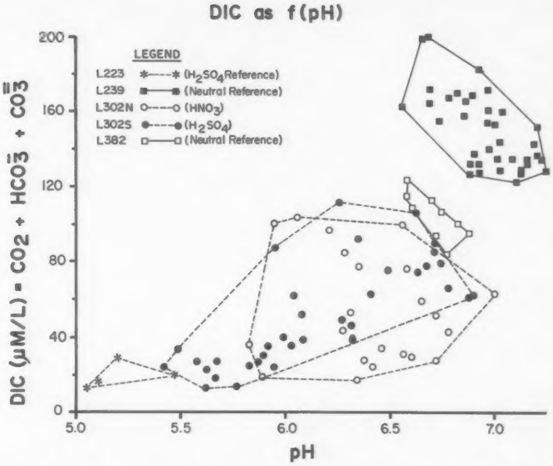

FIG. 2. DIC expressed as a function of pH. Observations for specific lakes are enclosed within envelopes.

L302N, the nitric acid basin, was acidified to a lesser extent than L302S (Fig. 1a), due to biological reduction of NO_3^- and associated alkalinity generation (Schindler 1987). Levels of nitrogen increased due to the addition of nitric acid, and SO_4^{-2} increased due to downstream transport from L302S. Greater epilimnetic phytoplankton production and biomass in the north than in the south basin (chlorophyll in N > S, Table 1; DeBruyn et al. 1982; DeBruyn 1984) may partly account for DIC being lower than would be expected due solely to lowered pH (Fig. 1b).

Methods

Carbon Metabolism in the Middle Littoral Zone

The criteria used to select sampling sites within each study lake were as follows: (1) location on flat surfaces of rock shelves or large boulders of similar slopes (<10 degrees) below the wave zone (≥1 m), (2) south facing slopes, but (3) no influence by an inflow. In midsummer of 1982, we moved the site in L239 about 200 m west to permit inclusion of large rock shelves similar to those sampled in L302N and L302S.

We sampled the periphyton of L239 and L302S at least once every 4 wk during the openwater period from midsummer of 1981 to the fall of 1984. L302N was sampled only three times in 1981 and five times in 1982; L382 was sampled only in 1984.

We have described the general strategy for monitoring epilithic periphyton in Turner et al. (1983). We selected in situ monitoring of natural populations rather than laboratory examination of samples on artificial substrata because of difficulty in completely retrieving the loosely attached component of the periphyton.

The sampling site in each lake included three areas that varied horizontally in total from 10 to 30 m, and vertically

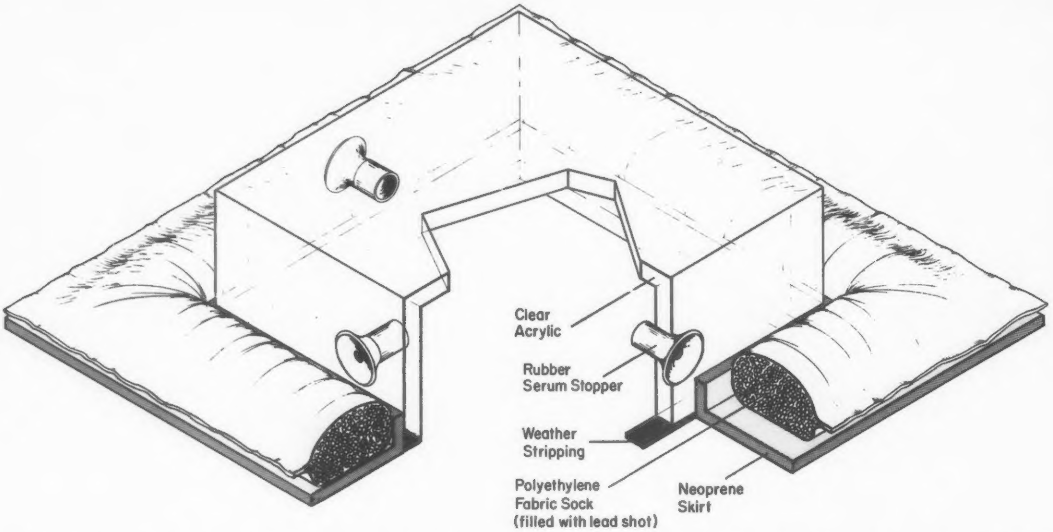

FIG. 3. Schematic diagram of an acrylic chamber used in the carbon metabolism studies. A SCUBA diver would carefully position the chamber, and place the polyethylene sock filled with lead shot on the neoprene skirt. This procedure isolated a periphyton sample with an overlying volume of water from which samples for DIC could be collected.

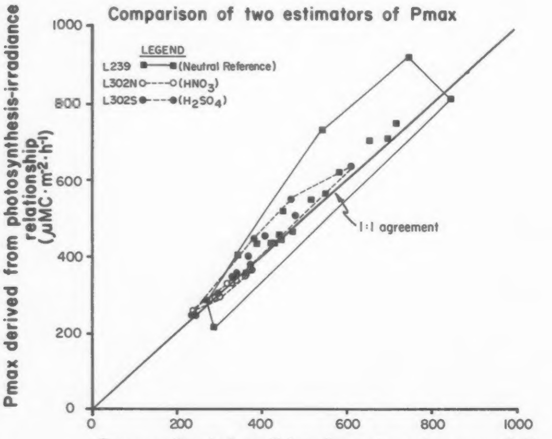

FIG. 4. Comparison of two techniques for estimating the maximum rate of gross photosynthesis ($P_{\max}$; slope = 0.84 ± 0.03 [SE], r^2 = 0.94, df, = 42). The rectangular hyperbolic estimate is based upon the relationship of $P_{\text{gross}} = (P_{\text{net}} + R_d)$ with irradiance. The second approach simply sums R_d and P_{net} measured at the maximum irradiance available.

from 1 to 2 m. When sampling we used SCUBA without fins to prevent disturbance of the periphyton. During each sampling and within each area divers would enclose three to four samples of bottom (100 or 200 cm^2 each) with acrylic chambers that had varying volume or light transmission characteristics. The volume of each regular volume chamber was 0.41 L. These chambers were open at the bottom, and sealed to the rock surface with an attached neoprene skirt upon which a polyethylene fabric sock filled with lead shot was placed (Fig. 3). Water exchange between chamber and lake was about 2%·h^{-1} (Turner et al. 1983), permitting correction of observed photosynthetic rates. We did not test for the potential effect of contamination by lead due to leakage. But we believe that such an effect would be small if it existed given that the rates we observed were similar to rates obtained using a lead-free technique (e.g., Turner et al. 1983).

Chambers made of black (0% irradiance) and clear acrylic (100% of available irradiance) allowed determination of dark respiration and net photosynthesis, respectively. We also restricted ambient light to some samples by using neutral density filters made of translucent acrylic. Two levels of attenuation of the light available at depth (I_z) were used: 48% (1981 to 1984) and 14% (1982 and 1983). Comparison of photosynthetic rates between paired 100% and 48% I_z chambers indicated whether the community was light saturated at 48% of I_z. Combining observations made at all light levels (0, 14, 48, and 100% I_z) allowed us to examine photosynthesis-irradiance relationships.

The techniques used to monitor DIC changes in the water overlying enclosed benthic samples during 1.5 to 4.5 h incubations, and the estimation of mean irradiance received by samples during incubation are described by Turner et al. (1983). Similarly, procedures for calculation of the community processes, dark respiration (R_d, µMC·m^{-2}·h^{-1}), and net photosynthesis (P_{net}, µMC·m^{-2}·h^{-1}) from changes in DIC in water overlying darkened and irradiated samples, respectively, are presented elsewhere (Turner et al. 1983).

To estimate algal photosynthesis without interference from production of DIC by community respiration, we assumed respiration was independent of light intensity and calculated gross photosynthesis (P_{gross}, µMC·m^{-2}·h^{-1}) as $P_{\text{gross}} = P_{\text{net}} + R_d$. This assumption is considered in detail by Graham and Turner (1987).

Before 1984 we estimated the maximum rate of gross photosynthesis ($P_{\max}$, µMC·m^{-2}·h^{-1}) from the relationship of gross photosynthesis to irradiance, incorporating observations made at 0, 14, 48, and 100% of available light. These photosynthesis-irradiance curves were well described mathematically ($r^2 = 0.98$, $n = 40$) by a rectangular hyperbola (Turner et al. 1983). Such estimates of P_{max} were independent of the irradiance received by samples during incubation. There was also good agreement between these values of P_{max} estimated from the equation for a rectangular hyperbola and those estimated by simply summing R_d and P_{net} at 100% of I_z (Fig. 4). The slope of 0.84 for the relationship suggested that the simpler approach underestimated P_{max} by about 16% (1 minus 0.84). Consequently, all estimates of the maximum rate of gross photosynthesis reported here as P_{gross} are the sum of dark respiration and net photosynthesis in chambers incubated at 100% of available irradiance.

We verified that samples were light saturated in 1984 by comparing P_{net} of samples receiving 100% of I_z with samples receiving only 48%. On five of 28 occasions P_{net} of 48% samples was less than in paired 100% samples ($P < 0.05$). Although the 48% samples were occasionally undersaturated, the underestimation of P_{max} was not substantial.

To examine the photosynthetic use of light, we analyzed the photosynthesis–irradiance observations made in 1982 and 1983. By analogy with nutrient uptake kinetics (Healey 1980), $I'' = P_{max}/I'$, where I' is a half-saturation parameter, can be interpreted as a measure of the efficiency of the photosynthetic utilization of light at low irradiance (Turner et al. 1983).

To ensure that photosynthesis in irradiated samples was not artificially nutrient-limited during incubations, we compared rates observed among paired regular (0.41 L) and oversize (0.80 L) chambers independent of any changes in irradiance during incubation. If DIC was limiting photosynthesis, we anticipated observing higher rates in chambers with twice the ratio of water to sample area. Therefore, higher photosynthesis in oversize chambers than in regular volume chambers (paired *t*-test, $P \leqslant 0.05$) would indicate nutrient limitation. In such instances, we used the photosynthetic estimates of the oversize chambers only. Using 4 h incubations in 1983, we noticed limitation only twice during eleven occasional checks. In 1984 we performed the checks routinely and reduced our incubation times from about 4 h to 1.5–2.5 h. Limitation occurred on four of 28 occasions in 1984.

In anticipation that as the pH declined, low DIC might limit photosynthesis during incubations, we routinely added DIC to samples in the acidified lakes (L302N and L302S) during 1982 to prevent nonlinearity of carbon uptake; however, we discovered subsequently that this procedure stimulated photosynthesis. Therefore, observations made with supplementary DIC have been excluded, except where noted.

Stock solutions of DIC were made up either with $NaHCO_3$ dissolved in distilled water, or by bubbling CO_2 gas through distilled water to yield dissolved CO_2 and H_2CO_3. Aliquots of these solutions were injected into sample chambers subsequent to emplacement, and mixed with water enclosed within the chamber. Samples for initial DIC concentrations were then taken from each chamber. Because we expected exchange with surrounding water at a rate of 2% h^{-1} (Turner et al. 1983), we adjusted rates of photosynthesis downward accordingly.

Algal Composition at Carbon Metabolism Sampling Sites

At each sampling a diver collected 5 cm^2 of material from each of the three areas within the site. A scraper similar in design to that described by Loeb (1981) was used. Because the sample suspension could include loosely attached material from the area adjacent to the scraper, beginning midway through 1983 we removed loose material surrounding the scraper prior to sample collection. Thus, areal concentrations which we report for algal density, are semiquantitative, and are probably overestimates in 1981 and 1982.

The three sample suspensions (about 50 mL each) were first stored in ice and darkness, and then within 6 h combined and diluted with distilled water to a concentration of 0.05 $cm^2 \cdot mL^{-1}$ (area scraped$\cdot$$mL^{-1}$). While lake water comprised about half of the volume of the final suspension, its contribution to algal density was negligible (<5%).

Forty mL of the particulate suspension was preserved by addition of 1–3% of acid Lugol's solution. Using a Sonifer Cell Disruptor (Model W140, Heat Systems–Ultrasonic Inc.) equipped with a microtip, samples were sonicated for two 15-s intervals at a frequency of 20 kHz. One or 2 mL aliquots were removed, stained with Fast Green FCF (Fisher Scientific Co.), and allowed to settle overnight. Algal cells were identified, usually to genus, and the sample enumerated at 400-power until 100 viable cells or filaments (100 μm lengths) of the dominant taxon had been observed. Living and "dead" cells were distinguished by the presence of cellular structures stained with FCF (Owen et al. 1978).

Algal species were grouped taxonomically according to Hustedt (1930), Patrick and Reimer (1966), and Findlay and Kling (1979). Areal densities of algae were calculated as:

$$\frac{\text{number of algae counted}}{\text{proportion of aliquot counted}\cdot\text{mL of suspension settled}} \times \frac{\text{mL of suspension collected}}{\text{m}^2\text{ scraped}}$$

The dominance value of each algal group was determined for each sample by assigning a value of 1 to the number of cells or 100 μ filaments of the dominant group in that sample. Numbers observed for other groups were scaled proportionately. A mean annual dominance value of 1.00 indicated that a member of that group dominated the lake site on all sampling occasions.

Shoreline Mapping of Filamentous Algae

In the littoral zone of each lake, all visible occurrences of filamentous algae on rock and log surfaces and on macrophytes were mapped in September from 1982 to 1984; however, we describe here only the major patterns in terms of biovolume. Observers in a boat estimated algal abundance as the fraction of total shoreline length covered with filamentous algae. Rock and log surfaces were present throughout the littoral zones of the study lakes, while macrophytes were variably and less extensively distributed. For each occurrence of filamentous green algae, observers estimated depth distribution (upper and lower limits), and growth thickness using a sounding line, rule, and acrylic-bottomed viewing bucket. We define "depth distribution" as the zone within which most, but not necessarily all, of the algal biomass occurred. For each occurrence, upper and lower depths were defined as the points at which significant reductions in growth thickness occurred, even though algae often extended to shallower and deeper depths in less abundance. All measures of algal depth and growth thickness were weighted, by shoreline length of occurrence, and averaged for each lake.

In the field, each major pattern was evaluated for consistency of taxonomic composition using a Swift FM-31 field microscope. A minimum of three samples of each pattern was also collected for later detailed taxonomic analysis. About 5 mL of each algal sample was placed in a 25 mL vial. The vial was then filled with lake water and 0.5 mL of acid Lugol's solution added to preserve the sample. Formalin (0.1 mL) was added later for long-term preservation. Subsamples were analyzed with a light microscope at 400× magnification to identify the algal filaments to genus. The percent contribution of each genus was estimated in terms of biovolume (at 150×) measured on duplicate subsamples.

Results

Carbon Metabolism in the Middle Littoral Zone

Dark respiration (R_d) showed a strong seasonal pattern in the neutral reference lakes (L239 and L382), with a maximum in midsummer and minima in spring and fall (Fig. 5a). There was a strong positive correlation of R_d with temperature (Table 2). No year-to-year trend was observed in R_d in L239 from 1981 to 1984 ($P > 0.5$). Dark respiration in both neutral reference lakes was of similar magnitude in 1984 (Fig. 5a, Table 3).

A seasonal pattern of R_d similar to that observed in the neutral reference systems occurred in both recently acidified basins; R_d was also positively correlated with temperature (Table 2). In spite of low water temperatures, R_d in the sulfuric acid basin (L302S) was elevated relative to other lakes in the fall of 1983 and 1984 (Fig. 5). Also, there may have been a trend towards increasing R_d in L302S from 1981 to 1984. This trend was less distinct in the nitric acid basin (r of $R_d = f(\text{year})$ for L302N and L302S was 0.30 and 0.47, respectively). In 1981 and 1982, values of R_d in L302S and L302N were similar to or less than those in L239, but by 1984 rates exceeded those seen in both L239 and L382 (Table 3, Fig. 5). The occasional observations made of R_d in the acid reference lake, L223, were similar to those made at comparable times in L302S.

Net photosynthesis (P_{net}) varied seasonally in the neutral reference basins (Fig. 5a), and was positively related to epilimnetic temperature (Table 2). In addition, P_{net} increased from 1981 to 1984 in L239 (Fig. 5a; $P_{net} = f(\text{year})$: $r = 0.42$, $n = 37$, $P < 0.01$). We wondered whether the decline in duration of incubation from 4 h to ≤ 2 h during the study could be responsible for this increase. We tested this possibility in L239 in 1984 under conditions favourable for nonlinear uptake: the highest epilimnetic temperature of the study, the lowest DIC of the year, and the highest P_{net} of the study. No difference was observed either between rates in samples enclosed by oversize and regular volume chambers incubated for 1.5 h, or between 1.5 h and 4.0 h incubations with regular volume chambers. The ratio of 4.0 h to 1.5 h rates was 1.01 ± 0.09 (paired t-test, $P > 0.05$, $n = 3$ pairs).

The relationship between P_{net} and temperature in the recently acidified basins was different from that in the reference lakes (Fig. 5b, Table 2). More importantly, P_{net} was positively related both to pH (Fig. 6a) and to ambient levels of DIC (Fig. 6b). We concluded that DIC was the controlling factor because P_{net} was stimulated by experimental additions of DIC (Fig. 7). Stimulation occurred whether DIC was added as $NaHCO_3$ or H_2CO_3 (Table 4), which had opposite effects upon initial pH. A check of the effect of increased DIC (160 vs 80 $\mu M \cdot L^{-1}$) upon R_d in L302S indicated no effect ($P > 0.1$, paired t-test, $n = 3$ pairs). Furthermore, our examination of linearity of carbon uptake indicated that increases in P_{net} were not artifacts of incubation-induced nutrient limitation. Thus, the lack of a positive relationship of P_{net} with temperature in the recently acidified basins (Table 4) is explained by the inverse relationship between DIC (specifically CO_2) and temperature (DIC $= f(\text{temperature})$: for L302S $r = -0.37, n = 38$, $P < 0.05$; for L302N $r = -0.81$, $n = 24$, $P < 0.01$).

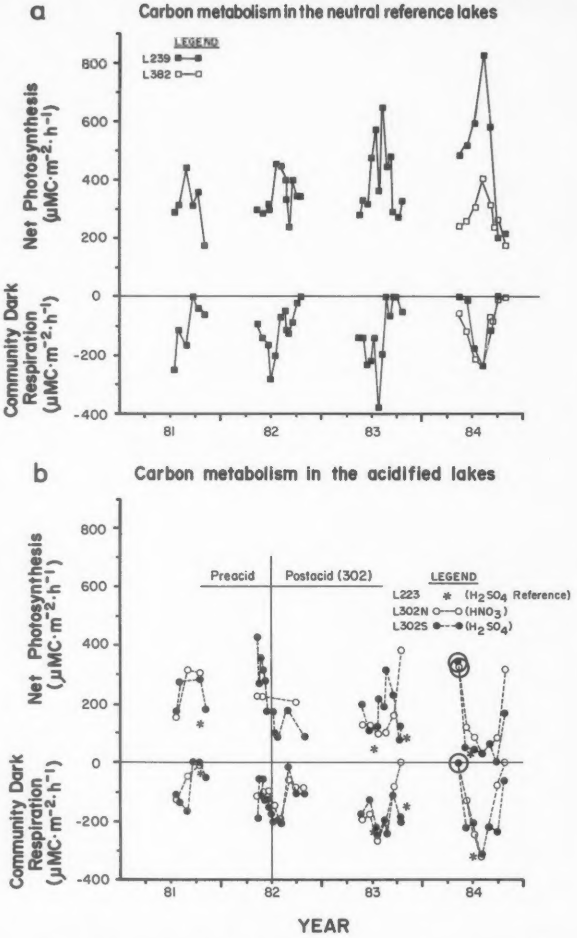

FIG. 5. Dark respiration (R_d) and net photosynthesis (P_{net}) during the openwater season at sampling sites in the two neutral reference systems (a: L239 and L382), and in the acidified systems (b: L223, L302N and L302S). Sampling began in July 1981. Each value represents the mean of three to nine observations. Circled values represent occasions when sampling occurred prior to the first addition of acid in 1984.

In contrast, there was no positive relationship between P_{net} and ambient concentrations of DIC within the neutral reference basins (Table 4). This is not surprising given the greater overall concentrations of DIC and smaller proportional changes seen in the neutral reference basins (Fig. 1b).

TABLE 2. Net photosynthesis and dark respiration (μmol $C \cdot m^{-2} \cdot h^{-1}$) examined as a function of temperature (°C). The period of observation of each lake was 1981 to 1984 except for L382 (1984 only). Negative values of R_d were assumed to be a result of analytical error, and have been replaced with values of 0.1.

		Dark respiration				Net photosynthesis			
Lake	Treatment	P	r	n	Slope (SE)	P	r	n	Slope (SE)
239	Neutral	<0.01	0.63	37	10.1	<0.01	0.58	37	13.5
	Reference				(2.1)				(3.2)
382	Neutral	<0.01	0.93	8	12.2	<0.01	0.90	8	9.3
	Reference				(1.9)				(1.8)
302N	HNO_3	<0.01	0.76	24	10.9	<0.01	−0.70	19	−10.3
					(2.0)				(2.6)
302S	H_2SO_4	<0.01	0.57	37	7.6	>0.05	−0.22	31	− 3.7
					(1.9)				(3.0)
223	H_2SO_4	>0.05	0.72	4	13.0	>0.05	−0.67	4	− 5.6
	Reference				(8.9)				(4.4)

TABLE 3. Mean rates of dark respiration (R_d), net photosynthesis (P_{net}), and calculated gross photosynthesis (P_{gross}) observed at the sampling sites. All parameters are expressed as μmol $C \cdot m^{-2} \cdot h^{-1}$. Each observation of R_d and P_{net} represents a mean of three samples; except for P_{net} in 1984 where each observation may represent the mean of 3 to 9 samples. Values for 1981 represent late summer and fall only. Values of respiration < 0 were transformed as in Table 2.

			R_d			P_{net}			P_{gross}		
Lake	Treatment	Year	Mean	SE	N	Mean	SE	N	Mean	SE	N
239	Neutral	81	105	38	6	315	36	6	420	57	6
	Reference	82	111	23	12	346	19	12	458	27	12
		83	130	33	12	400	36	12	530	53	12
		84	76	37	7	488	83	7	565	116	7
		81−84	106	11	4	387	38	4	493	33	4
382	Neutral	84	98	30	8	276	24	8	382	59	7
	Reference										
302N	HNO_3	81	62	35	3	256	51	3	317	22	3
		82	110	15	8	218	7	3	319	16	3
		83	154	39	6	164	44	6	318	20	6
		84	144	47	7	145	46	7	289	24	7
		81−84	118	21	4	196	25	4	311	7	4
302S	H_2SO_4	81	77	29	6	231	31	4	305	38	4
		82	132	15	15	221	34	11	348	41	10
		83	191	16	9	176	25	9	367	33	9
		84	181	41	7	99	45	7	277	17	7
		81−84	145	26	4	181	30	4	324	20	4

Although when concentrations of DIC were elevated experimentally by addition of $CO_2 + H_2CO_3$ or $NaHCO_3$, P_{net} was stimulated even in L239 (Table 4). Interestingly, the slope of $P_{net} = f(\text{DIC})$ was greater in both sulfuric acid basins (L223 and L302S) than in either L239 or L302N.

The seasonal pattern of P_{gross} in the neutral reference basins (Fig. 8) was positively related to temperature (L239, $r = 0.75$, $n = 37$, $P < 0.01$; L382, $r = 0.97$, $n = 8$, $P < 0.01$) which is not surprising given that both R_d and P_{net} were similarly but less strongly related to temperature. P_{gross} may have increased in L239 from 1981 to 1984 (Fig. 8: $P_{gross} = f(\text{year})$: $r = 0.28$, $n = 37$, $P < 0.1$) reflecting primarily the increase in P_{net} (Fig. 5). In addition, P_{gross} was greater in L239 than in L382 during 1984 (Fig. 8). Given the similarity in both R_d and epilimnetic temperatures in the two lakes, differences in P_{gross} were probably due to the stimulatory effect of higher DIC levels on P_{net} in L239.

There was no recurring seasonal pattern of P_{gross} evident in the recently acidified lakes (Fig. 8). Overall P_{gross} was independent of temperature ($P > 0.1$), because R_d was proportional to temperature, while P_{net} was unrelated or negatively correlated (Table 2). Therefore, the inverse correspondence between DIC and temperature (Fig. 1b), and the positive relationship between P_{net} and DIC (Fig. 6b) obscured any positive relationship of P_{gross} with temperature.

The seasonal pattern of the efficiency of photosynthetic utilization of light at low irradiances ($I'' = P_{max}/I'$) was similar in 1982 and 1983 in L239. The normalized half-saturation constant decreased from spring to late summer, and increased rapidly in fall. Values of I'' in L302S during the

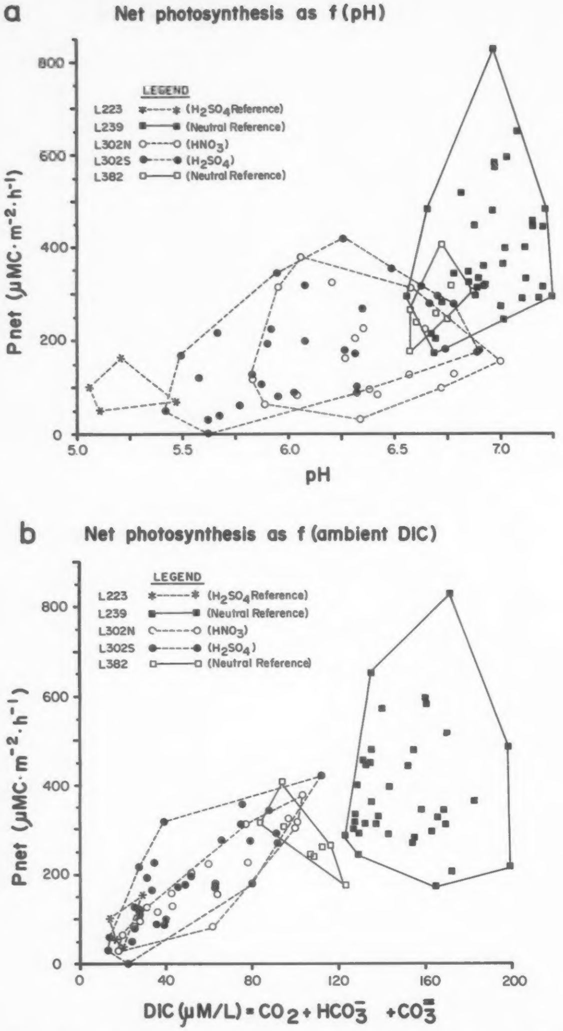

FIG. 6. Net photosynthesis (P_{net}) expressed as a function of pH (a) and DIC (b). All values for a specific lake are enclosed within an envelope. Each value represents the mean of three to nine observations.

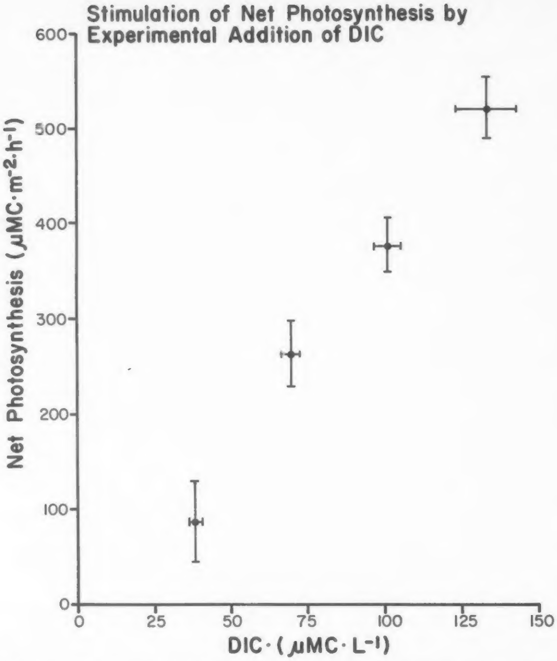

FIG. 7. An example (L302S, July 1982) of stimulation of net photosynthesis by experimental addition of DIC. The standard deviation of three replicates is shown at each combination of photosynthesis and DIC.

spring of 1983 were lower than those in L239, although higher than values observed during the preacidification period of 1982. Because there were fewer data for L302N, there was no clear pattern, but values were more similar to those measured in L239 than in L302S. As a consequence, no effect of acidification on I'' was apparent (Table 5). I'' did vary approximately with the attenuation coefficient. Mean values of I'' (Table 5) in L302S were lower than in either L239 or L302N; while mean values of the attenuation coefficient (m^{-1}) were 0.53 in L302S, 0.68 in L239 and 0.67 in L302N.

Algal Composition at Carbon Metabolism Sampling Sites

In epilithic periphyton below the wave zone, the seasonal pattern of change in algal composition varied among lakes. In the neutral reference basin, L239, diatoms (*Achnanthes minutissima* Kütz, *Navicula incerti* Grun) dominated in the spring, while a shift to filamentous blue-green algae (*Lyngbya*) occurred by midsummer. In the remaining lakes, subwave zone communities were dominated by filamentous Cyanophyceae (*Lyngbya*, *Anabaena* and *Oscillatoria*) throughout the ice-free season (Table 6). Chlorophyceae were unimportant in L239 and L382 (dominance values ≤ 0.1), although *Mougeotia* was occasionally seen. In contrast, Chlorophyceae, including *Mougeotia*, were characteristically more common than diatoms in both L302N and L302S.

Shoreline Mapping of Filamentous Algae

Filamentous epilithic algae (i.e. on rocks and logs) were entirely absent in circumneutral L239 (Table 7). Epiphytic growth was almost entirely absent from L239 in 1982 and 1983, but covered 28% of the shoreline in 1984. The epiphytic community was dominated by a mixture of green algae in 1982, and by *Mougeotia* in 1983 and 1984.

Development of filamentous algae was not mapped as intensively in L382 as in the other lakes, although results from brief examinations in 1983 and 1984 by surface observers were consistent with those from L239. That is, growth in L382 was always absent on rock and log surfaces and present on macrophytes near an acidic bog seepage, while in 1984 macrophytes were more extensively colonized.

Filamentous epilithic algae occurred along the entire shoreline of L223 in all years, while epiphytic coverage was relatively constant at 31–46%. Epilithic growth was present

TABLE 4. Net photosynthesis as a function of DIC. The relationship of P_{net} (μmol C·m^{-2}·h^{-1}) with DIC (μmol C·L^{-1}) is presented both for ambient levels only, and for ambient and experimentally elevated DIC. The period of observation extends from midsummer 1981 to fall of 1984 for all lakes but L382 (1984 only). Each value of $\underline{n}$ represents the mean of several observations as in Table 3.

		Ambient DIC						Elevated and ambient DIC				
						DIC						
Lake	Treatment	P	r	n	slope (SE)	Min	Max	P	r	n	slope (SE)	DIC Max
239	Neutral Reference	>0.05	0.05	37	0.34 (1.13)	123	200	<0.01	0.50	42	2.32 (0.63)	285
382	Neutral Reference	<0.05	−0.79	8	−4.07 (1.29)	84	124					
302N	HNO_3	<0.01	0.93	19	3.26 (0.32)	18	104	<0.01	0.95	30	3.95 (0.26)	287
302S	H_2SO_4	<0.01	0.82	31	3.32 (0.42)	13	112	<0.01	0.90	53	5.06 (0.34)	258
223	H_2SO_4 Reference	>0.05	0.60	4	5.05 (4.75)	14	29	<0.01	0.99	9	4.85 (0.31)	174

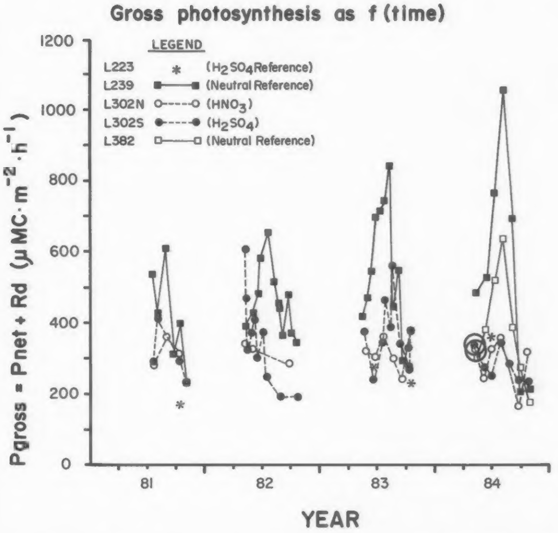

FIG. 8. Time course of gross photosynthesis ($P_{gross} = P_{net} + R_d$) in all basins monitored. Circled values represent occasions when sampling occurred prior to the first addition of acid in 1984.

in greatest abundance from just above the waterline to about 1.0 m depth, while epiphytic growth was prevalent from the waterline to 0.6 m depth. *Mougeotia* dominated in all occurrences. Epilithic growth thickness was about 1 cm, while epiphytic growth ranged from 1 to 2 cm in thickness. Shoreline coverage and growth thickness in L223 was noticeably greater than in all other lakes.

The amount of shoreline in L302S affected by filamentous algae increased markedly from 1982 to 1983. This response was primarily due to a major increase in epilithic coverage (from 10% in 1982 to 100% in 1983 and 1984). Moreover, the depth distribution of significant epilithic growth deepened from a narrow waterline band in 1982, to a maximum depth of about 1 m by 1983. Although *Mougeotia* was always a dominant alga (19−72%), *Spirogyra* (another member of the filamentous Zygnematales) also dominated in 1984. Epiphytic coverage, which was shallow (< 0.5 m), increased from 46% in 1982 to 63% in 1984. *Mougeotia* dominated this community in all years (49−69%).

TABLE 5. Interlake variation of $I'' = P_{max}/I'$ during 1982 and 1983. Estimation of I' and P_{max} is described in the text. The parameter I'' is equivalent to the efficiency of photosynthetic utilization of light at low irradiances. The number of series of photosynthesis−irradiance observations is n (each series was usually comprised of 10 to 12 photosynthesis−irradiance pairs).

			I'' (mmol C·Einstein^{-1})	
Lake	Treatment	n	Mean	SE
239	Neutral Reference	24	6.7	2.0
302N	HNO_3	9	8.6	1.8
302S	H_2SO_4	12	3.7	0.8

Epilithic coverage of the L302N shoreline was always low, about 8% from 1982 to 1984. Growth consisted of a sparse waterline band in 1982 and 1983, while in 1984 coverage deepened to 0.3 m. *Spirogyra* was the dominant epilithic alga (51−100%) in all years. In contrast, epiphytic coverage was variable: widespread in 1982 (covering about one-half of the shoreline), completely absent in 1983, and present again in 1984. The 1982 and 1984 communities were dominated by blue-green (i.e. *Scytonema*) and green algae (*Zygogonium* and *Mougeotia*), respectively.

Discussion

Carbon Metabolism in the Middle Littoral Zone of the Sulfuric Acid Lakes

Carbon metabolism of epilithic periphyton below the wave zone (≥1 m) was affected early in the experimental acidification of L302S (pH ≥ 6.0). Specifically, net photosynthesis (P_{net}) declined (Fig. 5), refuting the hypothesis of Almer et al. (1978) that periphyton productivity in general would be

TABLE 6. Algal density, number of taxa identified, and taxonomic composition at the sampling sites. Densities and dominance were determined as described in the text. Estimates of density are semiquantative, with 1981 and 1982 values less reliable than in later years. Values for 1981 represent samples collected only in late summer and fall.

				Algal density ($10^4 \cdot cm^{-2}$)	Number of taxa	Dominant algal groups		
Lake	Treatment	Year	*N*	Mean ± SD	Mean ± SD	Cyano	Chlor	Diato
239	Neutral Reference	81	7	179 ± 92	21 ± 3	1.00	0.10	0.25
		82	6	280 ± 85	12 ± 3	0.70	0.05	0.60
		83	12	127 ± 50	14 ± 4	0.82	0.03[a]	0.45
		84	7	175 ± 36	16 ± 5	0.75	0.06	0.87
382	Neutral Reference	84	7	141 ± 39	14 ± 5	1.00	0.10	0.22
302N	HNO_3	81	2	121 ± 81	16 ± 8	1.00	0.13	0.22
		82	6	392 ± 245	10 ± 3	1.00	0.15	0.06
		83	5	111 ± 21	9 ± 4	1.00	0.10	0.06
		84	7	127 ± 44	12 ± 4	1.00	0.19	0.07
302S	H_2SO_4	81	6	173 ± 72	13 ± 6	1.00	0.06	0.10
		82	6	402 ± 129	11 ± 5	1.00	0.13	0.08
		83	6	137 ± 67	8 ± 4	1.00	0.06[a]	0.06[a]
		84	8	83 ± 21	15 ± 5	0.99	0.27	0.25
223	H_2SO_4 Reference	81	1	115	11	0.35	0.06	1.00
		83	2	103 ± 11	13 ± 4	0.73	0.61	0.24

[a] Other algal groups were found to be more important. For L239 in 1983, the value for Chrysophyceae was 0.09; and for L302S in 1983, Peridineae had a value of 0.16.

enhanced as a consequence of acidification. We postulate that P_{net} declined with acidification in direct response to lowered DIC (Fig. 6, 7; Table 4), and indirectly to increased H^+, because additions of bicarbonate or carbonic acid, which have opposite effects upon pH, stimulated P_{net} similarly. The conclusion that DIC controls P_{net} in low DIC lakes is consistent with the observation by Sheldon and Boylen (1975) that bicarbonate addition stimulated ^{14}C uptake of diatom-dominated epiphytic periphyton in a circumneutral oligotrophic lake.

Dissolved inorganic carbon (DIC) declined (Fig. 1b, 2) as a consequence of the loss of HCO_3^- with lowered pH (Fig. 1a). A seasonal pattern in DIC concentrations was obvious in all lakes (Fig. 1b). Because CO_2 concentrations were independent of H^+ (Hutchinson 1957), higher DIC in spring and fall was due to greater solubility of CO_2 at low temperatures, and to entrainment of hypolimnetic DIC during spring and autumn overturns. The midsummer depression seen in the neutral reference lakes was accentuated in the acidified lakes by the H^+-induced loss of bicarbonate. We observed midsummer DIC values of 13 $\mu M \cdot L^{-1}$ (Fig. 1b), approximately the equilibrium concentration of atmospheric CO_2 in water. This was much lower than the mean seasonal value of 80 $\mu M \cdot L^{-1}$ prior to acidification (Table 1).

The general variation in P_{net} among the study lakes can be explained by differences in DIC (Fig. 6b). The trend in P_{net} from 1981 to 1984 in the recently sulfuric acidified lake (L302S) was the reverse of the trend in neutral L239. Whereas P_{net} declined by about one-half in L302S, there was about a 50% increase in L239 (Table 3, Fig. 5), possibly because of a corresponding increase in DIC (Fig. 1b; $P < 0.05$). A similar but unexplained increase in phytoplankton ^{14}C productivity was also seen in other ELA neutral reference lakes (Shearer et al. 1987).

The seasonal pattern of P_{net} in the neutral reference lakes (L239 and L382), was controlled in part by temperature (Table 2). Because temperature and DIC cycles were inversely related, P_{net} was inversely proportional or unrelated to ambient levels of DIC (Table 4). With the greater carbon limitation in the acidified systems, seasonal variation of P_{net} (Fig. 5b) was controlled largely by the seasonal variation of DIC (Fig. 1b). Although the seasonal pattern of P_{net} in L302S prior to H^+ additions was unclear (because of incomplete data collection during 1981), the cycle was probably similar to that of the neutral reference lakes, with magnitude equivalent to P_{net} in L382 (given the similarity of preacidification concentrations of DIC; Table 1). By 1984 an inverse pattern was established in L302S, with yearly maxima occurring when DIC was highest (Fig. 1b) at the beginning and end of the openwater season (Fig. 5b), in spite of low water temperatures.

Previous observations of periphyton productivity in acidified lakes are restricted to ^{14}C uptake, and are often complicated by inadequate experimental control of DIC. At saturating irradiances, ^{14}C uptake is likely to be greater than P_{net} (Turner et al. 1983). Müller (1980) concluded that ^{14}C uptake was independent of pH from his mesocosm in vitro studies of L223 periphyton. This result was probably an artifact of his methods in which "equal aliquots of scraped periphyton from each tube were added to bottles of filtered lake water....". Because actual DIC would have been very different among the experimental enclosures (pH 4.0 to 6.5; see Fig. 2), incubation in one water type would not have yielded "relative production in different tubes", given the strong relationship observed between P_{net} and DIC (Fig. 6b and 7, Table 4).

In Sweden, Lazarek (1982a) concluded that inorganic carbon was not limiting because epiphytic ^{14}C uptake in the acidified Lake Gärdsjön (pH 4.3–4.7) was similar to that in the limed Lake Högsjön (pH 6.3–6.7). But the levels of DIC which he reported for the acidified lake (averaging 100 $\mu M \cdot L^{-1}$) seem high, and may reflect methodological problems associated with either the experimental addition of $NaH^{14}CO_3$

TABLE 7. Shoreline development of filamentous algae observed during September surveys. (+ Indicates algae were above waterline at the time of observation; * Genus represented > 15% of filamentous algal biovolume; ** records do not distinguish between the epiphytic and epilithic pattern; however, observations indicated that values were similar to those in 1982; *** In 1983 and 1984, coverage was similar to that in L239).

				Weighted mean			
				Depth			
Lake	Treatment	Year	Percent shoreline covered	Upper (cm)	Lower (cm)	Thickness (cm)	Dominant algae (%)*
Epilithic Community							
239	Neutral Reference	82	0				
		83	0				
		84	0				
382	Neutral Reference	***					
302N	HNO_3	82	8	+ 5	0	<1	*Spirogyra* (51), *Zygnema* (25)
		83	9	+10	0	<1	*Spirogyra* (70), *Zygnema* (22)
		84	7	+ 5	30	1	*Spirogyra* (100)
302S	H_2SO_4	82	10	+ 9	1	<1	*Mougeotia* (67), *Zygnema* (29)
		83	100	+ 1	>100	1	*Mougeotia* (72)
		84	100	10	>100	<1	*Spirogyra* (62), *Mougeotia* (19)
223	H_2SO_4 Reference	82	99	+ 7	> 98	<1	*Mougeotia* (85)
		83	100	+ 8	> 97	1	*Mougeotia* (95)
		84	100	+ 8	> 98	1	*Mougeotia* (98)
Epiphytic Community							
239	Neutral Reference	82	2	7	37	<1	*Zygogonium* (32), *Hapalosiphon* (29), *Bulbochaete* (27)
		83	1	0	35	<1	*Mougeotia* (54), *Bulbochaete* (17)
		84	28	2	31	<1	*Mougeotia* (70)
382	Neutral Reference	***					
302N	HNO_3	82	52	10	49	<1	*Scytonema* (35)
		83	0				
		84	44	2	22	<1	*Zygogonium* (41), *Mougeotia* (21) *Hapalosiphon* (18)
302S	H_2SO_4	82	46	8	42	<1	*Mougeotia* (69)
		83	**	**	**	**	*Mougeotia* (69)
		84	63	2	33	<1	*Mougeotia* (49), *Oedogonium* (40)
223	H_2SO_4 Reference	82	46	1	45	2	*Mougeotia* (93)
		83	46	0	62	1	*Mougeotia* (79)
		84	31	0	35	2	*Mougeotia* (88)

of unknown specific activity, or the freezing and storage of DIC samples in vacutainers.

In Norway, Hendrey (1976) examined in vitro ^{14}C uptake by epilithic periphyton (diatoms and filamentous green algae) which had developed in experimentally acidified stream channels. Insufficient methodological details were given to evaluate the precautions taken for DIC control. While areal ^{14}C productivity was higher at lower pH, Hendrey concluded that ^{14}C uptake per unit of chlorophyll had declined with reduced pH.

There is little information available about the relationship of periphytic respiration to H^+ stress. In our investigation, dark respiration (R_d) of epilithic periphyton in the middle littoral zone (1−2 m) did not decline with acidification (Fig. 5b, Table 3). This result is consistent with the observation by Kelly et al. (1984) that rates of decomposition in L223 sediments with epipelic periphyton were unaffected by the decline in pH from 6.7 (1976) to 5.1 (1981). Neither finding supports the hypothesis that reduced microbial respiration would be responsible for an increase in periphyton biomass (Hendrey 1976; Hall et al. 1980). In fact, R_d may have increased as a consequence of acidification (Fig. 5b). R_d in L302S was higher in 1983 and 1984 than in 1981 and 1982; this was not true in L239 (Table 3). Moreover, respiration was higher in L302S than in either neutral reference lake by 1984, although rates were similar in 1981.

Beginning in 1982, we consistently observed that respiration remained higher in L302S during the fall than in other routinely monitored basins (Fig. 5). We have not investigated the cause(s) of this phenomenon, but we hypothesize that it may reflect heterotrophy by the blue-green algae.

We did not detect a dramatic decline in calculated gross photosynthesis (P_{gross}) as a consequence of acidification (Fig. 8) as occurred for P_{net} (Fig. 5b). We believe that this discrepancy is partly an artifact of the incorrect estimation of P_{gross}. Without a direct measure of respiration in the light (R_i), we assumed R_i was equal to R_d; i.e., that respiration was independent of irradiance. Actual P_{gross} is less than $P_{net} + R_d$ (Graham and Turner 1987). Respiration in the light was less than R_d in both L239 and L302S, although the factors governing variation in $R_l:R_d$ remain to be determined. The magnitude of this bias is greater for communities where $R_d:P_{gross}$ is large. In the sulfuric acid lake (L302S) P_{gross} was overestimated by about 30% when this bias was probably small (high P_{net} and low R_d).

Three lines of evidence support the conclusion that P_{gross} was disrupted by acidification. First, calculated P_{gross} increased each year from 1981 to 1984 in circumneutral L239, while P_{gross} in sulfuric acidified L302S steadily declined (Fig. 8, Table 3). Second, while P_{gross} was strongly related to epilimnetic temperature in both neutral reference lakes ($P < 0.01$), this was not the case for L302S ($P > 0.1$). Third, P_{gross} in L302S prior to the first addition of sulfuric acid in the spring of 1984 (Fig. 8) was comparable to P_{gross} in neutral L382. While P_{gross} increased with increased temperatures in L382, the pre-acidification, low-temperature observation was almost the highest seen that year in L302S.

Any increase in epilimnetic transparency during spring and early summer resulting from sulfuric acid addition (Shearer et al. 1987) probably had little effect on the efficiency of light utilization by epilimnetic periphyton below the wave zone. We found earlier (Turner 1981) that such epilimnetic periphyton were usually light-saturated at irradiances characteristic of the epilimnion during the openwater period. This was confirmed both by the small values of I'' ($= P_{max}/I'$; Table 5) and by comparisons between P_{net} of samples exposed to 48% and 100% of available light.

Carbon Metabolism in the Middle Littoral Zone of the Nitric Acid Lake

The effects of nitric acid on carbon metabolism of periphyton in the middle littoral zone are not yet clear. This uncertainty is due largely to the smaller decline in pH seen in the nitric acidified L302N (Fig. 1a; from 6.7 in 1981 to 6.2 in 1984) than in the sulfuric acidified L302S (5.6 in 1984). The increased transparency seen in spring and early summer in sulfuric acid basins (Shearer et al. 1987) was not seen with the addition of nitric acid (Shearer et al., unpublished data), so that the L302N water column was less transparent than L302S. The irradiance half-saturation parameter (I'') for periphyton in the middle littoral zone of L302N was also the largest of the three basins studied (Table 5), indicating most efficient photosynthetic use of light at low irradiances. The probable effects on benthic photosynthesis below the wave zone in the epilimnion are minimal given the usual status of light saturation. Moreover, an earlier modelling exercise (Turner 1981) indicated that variation of the half-saturation parameter had little impact on seasonal photosynthesis in the epilimnion.

As a consequence of lowered DIC, photosynthesis was disrupted in the nitric acid basin. While DIC declined substantially (Fig. 2), concentrations remained higher than in sulfuric acidified L302S during 1984 (Fig. 1b). The composition of the DIC system was also different during 1984, with CO_2 ranging from 25 to 75% of DIC in L302N, versus 70 to 100% in L302S (Turner and Linsey, unpublished data). As a consequence of the decrease in DIC, the P_{net} of periphyton in the middle littoral zone declined compared to the neutral reference lakes (Fig. 5), with seasonal variation appearing to be controlled by DIC (Table 4, Fig. 6b), rather than by temperature (Table 2). As in L302S, R_d did not decline with increased H^+ (Fig. 5b). But the seasonal pattern of R_d in L302N remained similar to the reference systems, without evidence of the elevated rates seen in the fall in L302S (Fig. 5b). In general, carbon metabolism of periphyton in nitric acidified L302N was intermediate between the neutral reference and sulfuric acidified systems.

Algal Composition and Abundance in the Sulfuric Acid Lakes

The most striking response of periphyton to acidification was proliferation in the upper littoral zone of filamentous green algae (hereafter referred to as fga) of the order Zygnematales, especially *Mougeotia* sp. (Table 8). Fga became established early in the acidification process as predicted from mesocosm experiments (Müller 1980). Accumulations of fga were composed of viable algae, consistent with other observations in Ontario (M. Jackson, unpubl. data). Although no shoreline mapping was done prior to acidification of L302, from experience with similar lakes elsewhere (M. Jackson, unpublished data), fga were probably rare before addition of H^+. During 1982 and 1983, fga in L239 and L382 (Table 7) were usually restricted to macrophytes located near low pH inflows and seepages. The increased occurrence of thin accumulations of *Mougeotia* sp. on macrophytes in these neutral reference lakes in 1984, may be symptomatic of the gradual decline in pH (7.1–6.8) which occurred since 1981, concurrent with drops in precipitation pH (Schindler and Linsey, unpublished data) and runoff pH (Bayley and Schindler 1986). In both sulfuric acid lakes, however, macrophytic growths generally occurred more consistently, had greater thickness and covered a greater proportion of the shoreline.

Prevalence of fga on submerged rock and log surfaces was unequivocally characteristic of the sulfuric acidified lakes (L223 and L302S, Table 8). While Stockner and Armstrong (1971) and ourselves (Table 6) observed fga (e.g. *Mougeotia*) on rock and log surfaces in circumneutral L239, they were never abundant. *Mougeotia* was seen as a waterline band on rock and log surfaces in the first year of acidification of L302S (1982, Table 7) when the pH was about 6.3 (Fig. 1a). Development on rock and log surfaces was more extensive in terms of depth and shoreline coverage in the second year when the pH was 5.9–6.0.

While our observations of carbon limitation of benthic photosynthesis (Fig. 6b, 7, Table 4) were restricted to the filamentous blue-green algal-dominated community in the middle littoral zone, we believe that the same factors are significant for fga. Consistent with Whitton (1970) and Horner et al. (1983), we propose that because fga extend beyond their substratum, they are affected beneficially by water movement (the boundary layer thickness near their cell membranes decreased, and rates of nutrient uptake are increased). Thus, fga would have a competitive advantage at obtaining DIC at otherwise growth-limiting concentrations. This logic may also explain the accumulation of fga on macrophytes; although the possibility that fga are also utilizing CO_2 respired by macrophytes cannot be ignored (Wetzel 1983).

The filamentous blue-green algae (hereafter referred to as fba) which dominated below the wave zone in our study (Table 6) were members of the families Oscillatoraceae and Nostocaceae (*Lyngbya*, *Anabaena*, and *Oscillatoria*). These algae occurred in the floc or loosely attached material found on rock surfaces of low slope. They were distinct from other fba such as *Scytonema* and *Tolypothrix* which were observed coexisting with fga on rock surfaces in the splash and wave zones. A similar cyanophycean benthic mat was observed in the acidic Lake Gardsjön by Lazarek (1982b), although the structure of this community was reticulate, and not flocculent. Interestingly, Lazarek observed photosynthetic rates of about 200 $\mu M \cdot m^{-2} \cdot h^{-1}$ at an "ambient" DIC of 80 $\mu M \cdot L^{-1}$, comparable to P_{net} of the fba community of our sulfuric acidified L302S when exposed to similar levels of DIC (Fig. 7).

We do not believe that fba developed in response to acidification because they were also dominant in the neutral reference lakes below the wave zone (Table 6). The importance of fba in unacidified lakes was unexpected because Stockner and Armstrong (1971) had concluded that diatoms were dominant (in terms of areal density) throughout the year in epilithic periphyton of ELA neutral reference lakes. While we found diatoms were most abundant in spring and early summer in L239, fba characteristically were dominant by mid to late summer. In L382, they dominated throughout the openwater period (Table 6). Although this discrepancy may be due to compositional changes during the decade between studies, we suspect that methodological differences are responsible. Periphyton on rocks of 10–20 cm diameter, were sampled in the earlier study, while we incorporated a wider range of sizes, including rock shelves (>10 m). In addition, Stockner and Armstrong collected samples as deep as 5 m without SCUBA, while we found it difficult to collect samples with the flocculent component intact even with SCUBA. Also, a substantial fraction of the diatoms in our samples were nonviable. Finally, we believe sonication of sample suspensions is essential for accurate quantification of fba, because many of the trichomes were incorporated into detrital clumps that were otherwise obscured.

In spite of descriptions of cyanophycean mats in acidic lakes (Hendrey and Vertucci 1980; Stokes 1980; Lazarek 1982b), we wonder about the continued persistence of the fba-dominated periphyton because respiration rates continued or increased in magnitude with acidification, while P_{net} declined (Fig. 5b, Table 3). Possibly its persistence reflects a heterotrophic ability (Saunders 1972) in combination with continued supplies of organic material from continuing planktonic photosynthesis (Shearer et al. 1987) and from allochthonous material. Fba also coexist with detritus, the degradation of which would yield CO_2 in an otherwise carbon-limited environment. If Brock (1973) was correct that many blue-green algae are stressed in habitats where the pH is less than 5, then continued acidification of L302S may further reduce the growth of fba. We believe that such reduced growth of fba will contribute to the progressive proliferation of fga. Because communities dominated by fba are able to buffer a decline in pH within 1 mm of the mat surface (Jorgensen et al. 1983; Sweerts et al. 1986) this would delay the influence of reduced water column pH.

Algal Composition and Abundance in the Nitric Acid Lake

Algal composition in nitric acidified L302N also supports the intermediate status between the neutral reference and sulfuric acid systems. Similar to the neutral reference systems (Table 6), fba remained dominant below the wave zone. In contrast to the extensive proliferation in the shallow littoral zone of L302S, fga were minimally present on a sheer rock face as a waterline band in 1982 and 1983, and detectable to 0.3 m in 1984 (Table 7). Interestingly, it was *Spirogyra* and *Zygnema*, and not *Mougeotia*, which dominated this pattern. Occurrence of fga as epiphytes was erratic, and taxonomic composition was more similar to the neutral reference lakes than to the sulfuric acid basins (Table 7).

Conclusions

1. The littoral zone mapping and metabolic components of this investigation measured community responses of filamentous green algae (fga) and filamentous blue-green algae (fba), respectively, and exclusively of one another. While fga were favoured by acidification, fba were adversely affected.
2. The hypothesis that increased periphyton biovolume may be explained by accumulation of nonviable material (Hendrey et al. 1976) was not supported by our field observations. Instead, periphyton accumulations in the artificially acidified basins were composed of viable fga (i.e. Zygnematales and especially *Mougeotia*).
3. Significant accumulations of fga were generally restricted to the upper littoral zone (i.e. weighted average maximum depth). This likely reflects high light requirements for optimal growth as exhibited by certain fga (Auer et al. 1983), but may also reflect enhanced uptake of DIC by water movement.
4. There is evidence from a number of sources that decreased grazing pressure (Hall et al. 1980; Økland 1980; I. J. Davies, personal communication) contributes to increased fga biovolume (Hendrey et al. 1976). Nevertheless, essential details on population densities and feeding rates of littoral zone invertebrates known to selectively crop fga have still to be determined. The importance of other growth controlling factors such as temperature, light, nutrients (e.g. DIC), and quite logically H^+, should also be determined, preferably with techniques comparable to those used by Auer et al. (1983).
5. Although the mechanisms are still unproven, two features of the development of fga on submerged rock and log surfaces make it useful as an indicator of H^+ stress in lakes. Firstly, presence of an organism is more definitive than its absence, because absence may be due to unrelated factors. Secondly, the development of fga occurs at relatively high pH (≥ 6.0), and therefore can serve as an inexpensive and early warning of acidification.
6. The hypothesis that growth of fba is enhanced by acidification (Lazarek 1980) was not supported by our findings. Instead, and consistent with Brock (1973), fba (i.e., predominantly *Lyngbya* and *Anabaena*) showed clear signs of metabolic stress as indicated by decreased P_{net}. Net photosynthesis was strongly correlated with DIC, where DIC was a function of pH, indicating that inorganic carbon was the primary nutrient limiting the growth of fba in acidified systems. This dependence of P_{net} on DIC implies, according to Raven (1970), that fba are able to utilize bicarbonate. If, in contrast, fga are better able to acquire free CO_2, which predominates at lower pH's, then fga would gain a competitive advantage with acidification (Raven 1970). Thus, the form as well as quantity of available inorganic carbon may directly contribute to the apparent opposite response of the fba and fga to acidification.

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Photoinhibition of Respiration in Epilithic Periphyton

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To determine whether respiration in the light was equal to respiration in the dark we examined epilithic periphyton from a lake acidified experimentally with sulfuric acid. Because of the low concentrations of dissolved inorganic carbon, we could use both ^{12}C and ^{14}C uptake techniques. Using the ^{14}C technique we could correct for residual photosynthesis in samples treated with the photosynthetic inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea). DCMU did not alter rates of dark respiration. However, respiration of DCMU-treated samples in the light was less than in the dark ($P < 0.01$). This photoinhibition of respiration was about 40% of dark control values. If we had calculated gross photosynthesis for the specific conditions of this experiment, but assumed incorrectly that light and dark respiration were equal, we would have overestimated gross photosynthesis by about 30%. Thus, if the ratio of respiration to photosynthesis is high, researchers will have to evaluate the effect of light on respiration to better estimate gross photosynthesis. The technique we describe, of monitoring both ^{12}C and ^{14}C flux in DCMU-treated samples in the light, will provide an underestimate of respiration in the light.

Les auteurs ont étudié le périphyton épilithique d'un lac acidifié expérimentalement à l'acide sulfurique afin de déterminer si la respiration était la même à la lumière qu'à l'obscurité. Étant donné les faibles concentrations de carbone inorganique dissous, il était possible d'utiliser les techniques d'assimilation du ^{12}C et du ^{14}C. L'utilisation de la technique au ^{14}C permettait d'apporter des corrections pour la photosynthèse résiduelle des échantillons traités par le DCMU ((dichloro-3,4 phényl)-3 diméthyl-1,1 urée), un inhibiteur de la photosynthèse. Le DCMU n'a pas eu d'effet sur les taux de respiration à l'obscurité. Les taux de respiration à la lumière des échantillons traités au DCMU étaient cependant inférieurs à ceux obtenus à l'obscurité ($P < 0.01$). Cette photo-inhibition de la respiration correspondait à environ 40 % des valeurs des témoins (obscurité). Un calcul de la photosynthèse brute dans les conditions de l'expérience, basé sur l'hypothèse incorrecte que les taux de respiration étaient les mêmes à la lumière qu'à l'obscurité, se serait traduit par une surestimation d'environ 30 %. Si le rapport de la respiration à la photosynthèse est élevé, les chercheurs devront donc évaluer les effets de la lumière sur la respiration afin d'améliorer leurs estimations de la photosynthèse brute. La technique décrite par les auteurs, qui consiste à contrôler en condition d'éclairement les flux de ^{12}C et du ^{14}C chez des échantillons traités au DCMU, se traduit par une sousestimation de la respiration à la lumière.

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In the past we developed laboratory and *in situ* methods for the study of productivity of epilithic periphyton[3] in soft water lakes (Turner 1981; Turner et al. 1983). In the study of the effects of sulfuric acidification on periphyton productivity of two small lakes (Lake 223 and Lake 302S) in the Experimental Lakes Area, northwestern Ontario (Cruikshank 1984), we observed that dark respiration accounted for a much higher proportion of gross photosynthesis than in neutral reference lakes (Turner et al. 1987). Because overestimation of respiration in the light would obscure the observed effect of acidification on gross photosynthesis (net photosynthesis adjusted for inorganic carbon supplied by respiration), it was critical to check our assumption that respiration occurs in the light at the same rate as in the dark.

The amount of respiration occuring in the light is still in question; estimates range from complete photoinhibition to photostimulation of respiration (the biochemical and physiological arguments are reviewed in Graham 1980; Raven and Beardall 1981). Much of the confusion arises because it is difficult to obtain simultaneous and accurate estimates of all parameters, especially R_i, in the photosynthetic equation:

$$(1) \qquad P_{\text{net}} = P_{\text{gross}} - R_i$$

where P_{net} = net photosynthesis,
P_{gross} = gross photosynthesis, and
R_i = respiration in the light.

Through the use of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a potent inhibitor of photosystem II (Sargent and Taylor 1971), we were able to measure respiration in the light independent of the reactions of photosynthesis. DCMU is believed to have no effect on respiratory metabolism in photosynthetic organisms (Sargent and Taylor 1971).

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[3] We use epilithic periphyton to mean the community found on rock surfaces, equivalent in meaning to "haptobenthos" (Hutchinson 1976). While algae are part of this assemblage, it also contains bacteria, fungi, zoobenthos, detritus and inorganic material.

The use of photosynthetic inhibitors usually involves the assumption that they completely shut down photosynthesis (e.g. Legendre et al. 1983). Such studies on the contribution of respiration to gross photosynthesis in the light have been done on higher plants and unialgal cultures (e.g. Ceulemans and Impens 1981; Bidwell 1977). These experiments on algal suspensions usually use 10^{-5} DCMU (e.g. Healey and Myers 1971; Legendre et al. 1983) to eliminate photosynthesis while leaving respiration intact. In pilot experiments (Table 1) we had assumed that such a dosage would also be effective for periphyton. But because there was measurable uptake of ^{12}C in the DCMU-treated samples in the light, we realized we would have to include a check for residual photosynthesis in our experimental design.

This study quantifies the effects of light on respiration rates in a mixed population (epilithic periphyton) under physiologically relevant conditions. We compared respiration by periphyton in the dark (R_d) to respiration in the light (R_i) in the presence of DCMU to estimate the error in P_{gross} introduced by assuming that $R_i = R_d$. Our experimental analysis included a correction for residual photosynthesis, which is usually lacking in other attempts to estimate the amount of respiration in the light.

Materials and Methods

The materials and methods employed in this experiment were generally based upon those described in Turner et al. (1983).

We used acrylic chambers (approximate interior dimensions of 10 cm wide, 20 cm long, 7 cm deep) to enclose samples colonized on either granite or vitrified clay plates (10 cm wide × 20 cm long; Turner 1981; Turner et al. 1983). Plates had been colonized with periphyton at approximately 1.5 m in Lake 302S for about 3 years. Samples were selected which had large and similar amounts of periphyton.

We removed the 12 samples from Lake 302S to a nearby laboratory where we randomly assigned samples to four experimental conditions: (1) irradiated (daylight) control, (2) irradiated DCMU, (3) dark control, and (4) dark DCMU. Each treatment had three replicates. From concurrent observations in Lake 302S we determined that the periphyton were likely light-saturated at the mean experimental irradiance of 110 $\mu E \cdot m^{-2} \cdot s^{-1}$. Samples were held within 2°C of the *in situ* temperature, 6°C.

DCMU Treatment and Sampling

All 12 chambers received an initial injection of 5 mL air to facilitate mixing. Partitioning of CO_2 between water and air phases did not cause a detectable error.

The six chambers designated "DCMU" (3 light, 3 dark) received a 1.0 mL injection of 4.3 mM DCMU in 50% ethanol; the final DCMU concentration in each chamber was 10^{-5} M. The six "control" chambers were given a 1.0 mL placebo injection of 50% ethanol. All chambers were then vigorously rotated for 20 seconds, which suspended the loosely attached component of the periphyton and ensured thorough mixing. After 0.5 h, we injected all irradiated chambers with approximately 1.0 mL of NaH $^{14}CO_3$-labelled epilimnetic water (about 0.04 $\mu Ci\ ^{14}C \cdot \mu mole\ DIC^{-1}$) and mixed the 12 chambers as before. Approximately 15 minutes later, we collected initial samples for DI ^{12}C and DI ^{14}C by syringe. Following 1.5 h of incubation, chambers were processed through a similar mixing-settling-sampling cycle. After sampling, syringes were sealed and stored in ice and darkness until analysis. Carbon-14 was not added to the dark chambers because in our experience with similar samples, dark ^{14}C uptake was usually less than 5% of P_{net} (Turner 1981; Turner et al. 1983), and may have represented reactions which do not occur in the light (Fogg 1974; Hecky and Fee 1984).

TABLE 1. Preliminary observations on the effect of 10^{-5} M DCMU treatment on ^{12}C flux ($\mu mol\ C \cdot m^{-2} \cdot h^{-1} \pm$ SD, $n = 3$) of periphyton in two lakes. In this pilot work, we made no attempt to measure residual photosynthesis. Negative values indicate net carbon release while positive values represent net carbon uptake.

		Irradiated		Dark	
Lake	Date	control	DCMU	control	DCMU
302N	18 Sept. 83	160 (±23)	21 (±51)	−84 (±70)	−79 (±25)
239	6 Sept. 83	480 (±103)	12 (±34)	−63 (±2)	—

Measurement of Photosynthesis and Respiration

Net photosynthesis and dark respiration were measured as a change in dissolved inorganic carbon (DIC) in water overlying the benthic samples (Schindler et al. 1973).

Initial and final DI ^{14}C activities in the water samples were measured as follows using a technique similar to that described by Shearer et al. (1985). Immediately after sampling, 2.00 mL ± 0.01 mL of water from each syringe were added to 150 μL of a carbon dioxide trapping agent (CO_2-mMET®, Amersham); then 15mL of PCS fluor (Amersham) were added. Mixed samples were dark-acclimated (>4 h) to eliminate photoluminescence and chemiluminescense, then counted in a Beckman LS2800 liquid scintillation counter to approximately 0.5% coefficient of variation using the "H number" method of quench monitoring (Horrocks 1977).

The concentration of DI ^{12}C in the water samples was determined by injecting 2.00 ± 0.01 mL of each into a sparging column with sufficient sulfuric acid to evolve the DIC as CO_2. This carbon dioxide was carried through a potassium perchlorate drying column and into an infrared gas analyzer using CO_2-free nitrogen gas (Turner et al. 1983; Herczeg and Hesslein 1984). All DI ^{12}C samples were analyzed within 3 h of collection.

Observed uptake of DI ^{12}C represents net uptake for an irradiated sample due to contribution of R_i, and was calculated using the following equation:

$$([DI\ ^{12}C]_i - [DI^{12}C_f] \times V/(A \times t) \tag{2}$$

where "i" and "f" subscripts refer to intial and final samples, "t" is the duration of incubation, "A" is the surface area of the sample, and "V" is the volume of water in the chamber. Units of DI ^{12}C uptake are $\mu mol\ C \cdot m^{-2} \cdot h^{-1}$. For a chamber incubated in darkness, in which there is net release of DI ^{12}C, equation (2) yields a negative number which is dark respiration (R_d).

Uptake of carbon measured by the ^{14}C technique is described by the following expression which is analogous to equation (2)

TABLE 2. Mean carbon fluxes (μmol C·m^{-2}·h^{-1}, ± SD, $n = 3$) for a 2 × 2 randomized block experiment performed in Lake 302S on 25 October 1984. Treatment levels are described in the text. Carbon-14 and ^{12}C estimates were obtained simultaneously, permitting estimation of residual photosynthesis and a corrected estimate of respiration in the light (R_i). Signs of fluxes are interpreted as in Table 1.

	Irradiated		Dark	
	control	DCMU	control	DCMU
12-C flux	137 (±18)	−37 (±6)	−170 (±63)	−141 (±7)
14-C flux	143 (±21)	51 (±16)	—	—
R_i	—	−88 (±15)	—	—

except that changes in ^{14}C activity in water are monitored rather than changes in DI ^{12}C:

$$(3) \qquad 1.05 \times [\mathrm{DI}^{12}\mathrm{C}]_i \times \{([\mathrm{DI}\ ^{14}\mathrm{C}]_i - [\mathrm{DI}\ ^{14}\mathrm{C}]_f)/[\mathrm{DI}\ ^{14}\mathrm{C}]_i\} \times V/(A \times t)$$

Units of DI^{14}C uptake are μmol C · m^{-2} · h^{-1}. We included an isotope discrimination factor of 1.05 (Strickland 1960).

To estimate R_i one must account for any $^{14}CO_2$ uptake observed in irradiated, DCMU-treated chambers. This $^{14}CO_2$ uptake can be interpreted as (1) residual DCMU-inhibitable photosynthesis persisting because of inadequate DCMU concentrations, or (2) DCMU-independent photosynthesis. From our experience in Lake 239, ^{14}C should measure carbon uptake primarily, while ^{12}C flux would reflect both uptake and release (Turner et al. 1983). Based on this assumption, we calculated respiration in the light (R_i) by correcting the ^{12}C estimate of respiration (equation 2) in irradiated DCMU samples for residual photosynthesis measured by ^{14}C uptake (equation 3).

Results and Discussion

Net fluxes of DI ^{12}C and DI ^{14}C for the irradiated control chambers were similar, although DI ^{14}C uptake was slightly higher (Table 2). The mean paired ratio of ^{14}C uptake: ^{12}C uptake in these irradiated controls was 111% ± 25% ($n = 3$ pairs). While this difference was within experimental error, such a bias would be predicted from our previous work (Turner 1981; Turner et al. 1983) in which we found that DI ^{14}C uptake usually was between DI ^{12}C-measured net photosynthesis and estimated gross photosynthesis. Because ^{14}C flux initially reflects only uptake of carbon, while ^{12}C measures the sum of uptake and release in short incubations, ^{14}C estimates of photosynthetic accumulation should be greater than those of ^{12}C.

The presence of measurable ^{12}C uptake in the irradiated DCMU-treated samples in the previous experiments (Table 1) prompted us to incorporate a ^{14}C test for residual photosynthesis in this experiment. Dark respiration was unaffected by 10^{-5} M DCMU, and substantial photoinhibition of respiration was observed with ^{12}C measurements (Table 2) confirming our original observations (Table 1). However, uptake of ^{14}C was seen in the irradiated DCMU chambers, indicating to us that DCMU inhibition of photosynthesis was not complete. We calculated that a residual photosynthesis of 35% ± 7% of the initial rate remained in the presence of DCMU (mean ± SD of the ratio of ^{14}C irradiated DCMU: ^{14}C irradiated control values, $n = 3$ pairs).

While the cause(s) of the residual carbon uptake is unclear, nonspecific adsorption of DCMU by organic matter may have lowered the effective concentration of this photosynthetic inhibitor in the periphyton mat. Unlike the algal suspensions reported in the literature, epilithic periphyton has a large and variable fraction of organic matter, both living and dead. Possibly, a more effective DCMU dosage should be determined for periphyton; however, its inherent heterogeneity may preclude the possibility of a completely inhibitory dosage which did not affect respiration. One can thus see the value of simultaneous measurement of ^{12}C and ^{14}C as an index of the effectiveness of DCMU.

A true estimate of respiration in the light may be calculated for DCMU-treated irradiated chambers by adjusting observed respiration (determined from DI^{12}C measurements) for residual photosynthesis (as calculated from DI ^{14}C data). In our experiment in acidified L302S (Table 2), calculated values of R_i were significantly different from measured R_d values ($P < 0.007$, factorial analysis of variance). True respiration in the light was approximately 60% of respiration measured in the dark (R_i = 63% ± 13% of R_d for dark DCMU samples, and R_i = 56% ± 18% of R_d for dark control samples). Consequently, photoinhibition of respiration was about 40%.

Calculation of P_{gross} using R_i data from this experiment produced an estimate of gross photosynthesis approximately 30% less than estimates based upon uncorrected R_d values. Our value for photoinhibition of respiration, and hence error in P_{gross} calculation, will not be a maximum because our experiment was performed at a relatively low water temperature (6°C); the contribution of respiration to calculated gross photosynthesis increases with temperature in acidified systems (Turner et al. 1987). We also have not accounted for CO_2 recycling within the algal cell (Raven 1972), which would diminish ^{14}C uptake observed in irradiated DCMU-treated samples. Hence, our estimate of the error in the calculation of P_{gross} is likely to be biased low.

Some, perhaps large, variability can be expected in the amount of photoinhibition. It will vary with ambient light conditions; this probably will not be a linear relationship (Scherer and Boger 1982). As well, seasonal changes in the composition of periphyton will influence the degree of photoinhibition. In controlled laboratory experiments with cultures of blue-green algae similar to those common in epilithic periphyton of both L302S and nonacidified ELA lakes (Stockner and Armstrong 1971; Turner et al. 1987), Scherer and Boger (1982) observed that photoinhibition of respiration ranged from 50% to 80% of dark control values.

While the degree of photoinhibition of respiration seen in this experiment cannot be used as a constant factor to correct other estimates of respiration for periphyton, it is a caution for future experimental design and interpretation of respiration of mixed populations.

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Perturbations in Zooplankton Communities in a Continuously Flowing Impoundment System Incubated in situ

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Holoka, M. H., and S. G. Lawrence. 1987. Perturbations in zooplankton communities in a continuously flowing impoundment system incubated in situ. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 154–162.

An apparatus which draws lake water, either filtered or unaltered, at a preestablished rate through four 40.75 L vessels incubated in situ is described. The system provides facilities for the synchronous addition of experimental liquids such as toxicants. All components are readily transportable. The system is self-powered for periods up to one month depending on the rate of flow chosen. This apparatus provides for the isolation of the zooplankton community from other compartments in the lake, incubation in natural conditions, experimental periods of from 1 to 30 d, maintenance of chosen concentrations of added materials continuously or non-continuously as appropriate, and entry of food organisms with concomitant dilution of wastes and metabolites.

The responses of selected zooplankton species or of zooplankton communities to impoundment, manipulation of predator or prey organisms and the addition of nutrient or toxic materials can be assessed as they occur in the naturally varying conditions of a lake environment while the population is being held as a separate and defined part of the whole-lake ecosystem.

Methods for the construction and use of the apparatus, and for collection of samples are described. Methods for enumerating organisms in several sizes of subsamples are assessed. Data generated in several experiments are analyzed using standard statistical methods and percent similarity indices.

Les auteurs décrivent un dispositif permettant de prélever de l'eau de lacs, filtrée ou non filtrée, à un débit prédéterminé. L'eau est amenée dans quatre enceintes de 40,75 L qui sont incubées in situ. Ce système permet aussi d'ajouter de façon synchrone certains produits, tels des toxiques. Toutes les composantes sont faciles à transporter. Le système est autonome pendant une période pouvant atteindre un mois, tout dépendant du débit choisi. L'appareil permet d'isoler la communauté zooplanctonique des autres compartiments du lac, d'effectuer une incubation en conditions naturelles, de réaliser des essais durant de 1 à 30 jours, de maintenir les concentrations désirées des produits ajoutés de façon continue ou discontinue et d'ajouter des organismes servant de nourriture tout en permettant simultanément la dilution des déchets et des métabolites.

Les réponses de certaines espèces ou communautés zooplanctoniques à la vie en enceintes, la manipulation d'organismes prédateurs ou proies de même que l'addition de matières nutritives ou toxiques peuvent être évaluées comme en conditions lacustres naturelles et variables tout en maintenant la population en tant que composante distincte et définie par rapport à l'ensemble de l'écosystème lacustre.

Les auteurs donnent le détail de la construction et de l'utilisation de l'appareil et décrivent le mode de prélèvement des échantillons. Ils évaluent des méthodes de dénombrement d'organismes de sous-échantillons, de tailles diverses et analysent, à l'aide de méthodes statistiques standard et d'indices de pourcentage de similitude, les données obtenues lors de divers essais.

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Studies of the impact of chemical and biological stresses on the various communities of aquatic ecosystems have usually been carried out either on single species under strict laboratory conditions or in naturally varying field conditions in which community components impinged upon each other. Much of the laboratory work has been channelled into the study of high concentrations of toxicants on a given size cohort of a single species. The effects of these studies are highly specific to the laboratory situation and are not readily extrapolated to the natural environment. Studies of processes in the natural environment usually involve the application of low concentrations of toxicants gradually applied over several years to the ecosystem as a whole. It is difficult to assign a change in any community component to a specific cause.

Intermediate in situ impoundment systems have been used to alleviate the problems encountered in these approaches. These include limnocorrals and small sealed incubators. A method for the examination of phytoplankton which includes exchange with the environment was developed by DeNoyelles and O'Brien (1974). We have designed a flow-through system for the in situ impoundment of zooplankton through which lake water may be pumped at a controlled rate.

The purpose of this paper is to describe the impoundment system and accompanying equipment. As well, several meth-

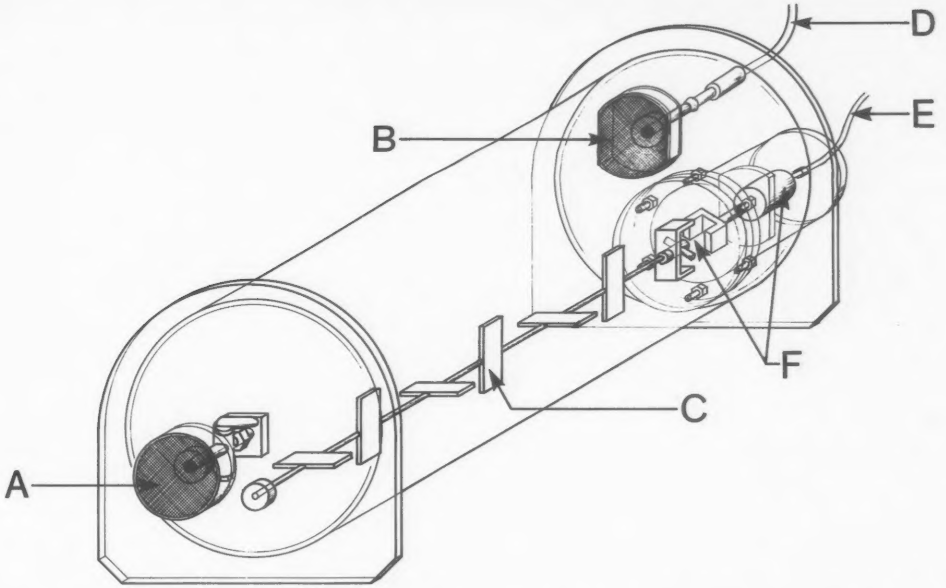


FIG. 1. Diagram of one of the four identical 40.75 L impoundment chambers. As the pumps draw water through the tube (D) connected to the internal filter (B), a negative pressure is created at the external filter (A) and lake water is drawn into the enclosure. Power cord (E) supplies current for the magnetic stirrer (F) which mixes the enclosed water volume by rotating the glass stirring rod and paddles (C).

ods for the enumeration of zooplankton are outlined and compared. The results of several experiments are presented and the reliability and variability of data are discussed. Various analytical methods are used to examine changes in zooplankton community relationships.

Methods and Materials

The Impoundment System

The impoundment vessels (Fig. 1) were constructed from extruded plexiglas tubing 34 cm in outside diameter, 68 cm in length, with a wall thickness of 6 mm. The tube ends were countersunk and glued into end plates of 9 mm thick plexiglas. This end plate extended 22 mm beyond the tube to provide an edge for mounting in a cage. The enclosed volume of each vessel was 40.75 L, chosen to be a reasonable upper limit with respect to weight and the dimensions of the system. This volume permitted detection of rare organisms and potentially reduced impoundment effects by minimizing the container surface to volume ratio (Pratt and Berkson 1959). A filter constructed of plexiglas and 54 μm nylon mesh (O'Brien and Kettle 1981) was placed on the inside of the vessel plate to prevent escape of the zooplankton (Fig. 1).

A magnetic stirrer, totally enclosed in plexiglas (Fig. 1) was mounted over a 10 cm diameter hole drilled in the vessel end plate. This stirrer assembly was removed when the vessel was cleaned and rinsed. The stirrer was magnetically coupled to an internal glass rod onto which six glass microscope slides had been glued. This rod rotated 8 cm from the bottom of the vessel at a nominal rate of 16 rpm. Mixing time was determined by adding dye and measuring the effluent with a spectrophotometer. No difference in absorbance was recorded after 10 to 15 min. The magnetic stirrer motors operated at 12 VDC and drew only 4 ma of current. The stirrers were operated from the same power supply as the pumps described below. This arrangement permitted a stirrer checking circuit to be inserted into the system. A small permanent magnet loudspeaker wired in series with one stirrer at a time, through a four-way switch, emitted a whirring noise caused by the commutation of the motor brushes. Visual confirmation of stirrer operation was often not possible when the system was submerged.

Four pumps drive the system. The pumping apparatus consists of two plexiglas box frames each supporting two syringe pump assemblies taken from a pipetting machine (Brewer). They are driven by a BOSCH 12 VDC 30 rpm gearhead motor. The pumping volume is adjusted by increasing or decreasing the eccentricity of the lower pivot which correspondingly affects the degree to which the plunger is drawn out of the barrel of the syringe. Once this is set, the pump ejects a constant and accurate volume during each revolution. The pump is not constantly driven but cycled on and off by a solid state low power timer. The manipulation of time delay and volume per stroke yields the final flow per hour. This method of cycling has several advantages. It greatly reduces power consumption, so that the battery does not require recharging during a 2-wk incubation when the system cycles once per minute. It also creates strong intermittant pulses of lake water flow which helps to draw in motile food organisms. In addition, wear is low. The pump motors and timer have performed over 500 000 operations without repair. The pump apparatus is equipped with an event counter which accumulates the number of strokes of the pump. Should the system stop operating, the volume of lake water pumped and the time of failure can be calculated. In addition to the main pumps, an auxiliary Cole Parmer peristaltic pump head (Cole Parmer, 7014) is attached in tandem at the back of the motor. These small volume pumps dispense the experimental liquid at a rate of 0.11 mL per revolution through plastic tubing (Tygon) into each treated experimental vessel. These ports for the injections are located on the end of the vessels 35 mm from the top, opposite the effluent filters. This arrangement provides adequate time for mixing of newly injected experimental liquid by the stirrers. Final vessel toxicant concentration is determined by manipulating the concentration of the toxicant (T_c) and by adjusting the ratio of volumes pumped by the main and auxiliary pumps. After an initial addition of toxicant is placed into the treated vessels, the concentration is maintained regardless of flow rate because all pumps operate in synchrony. The required concentration in the toxicant reservoir (T_c) is calculated by using the formula:

$$T_c = \frac{\text{Total pumped volume (mL)} \times \text{desired vessel experimental concentration}}{0.11}$$

Setup and Incubation of the System

To insure consistent results, the vessels had to be filled with lake water as uniformly as possible. A Pennak sampler (Pennak 1962) was used to obtain an integrated sample from the surface of the water to 4 m depth. The sampler was used to fill a 75 L plastic container placed in a boat. Originally this sample was divided between the four chambers and lake sample carboy using random draws with a 2 L beaker. Later, a sample divider was used. The divider consisted of a 17 cm diameter plexiglas cylinder, 60 cm in length suspended in gimbals to insure a vertical attitude in a rocking boat. The cylinder had ports placed in the sides at the bottom. Water flowed through equal lengths of 12 mm diameter thick walled Tygon tubing attached to the ports and into each chamber. A 22.9 L carboy was used to obtain a sample of the zooplankton present in the lake at the start of the experiment. It was filled concurrently with the vessels from the sample divider apparatus. Any patchiness of sample drawn from the 75 L reservoir was reintegrated in the divider apparatus and redistributed evenly. Use of this device reduced the filling time so that stress placed on captured zooplankton was reduced.

The final assembled apparatus (Fig. 2) consisted of four identical culture vessels clamped together by their ends in a cage assembly. A 25 mm diameter polyvinyl chloride (PVC) plastic plumbing pipe was screwed in perpendicularly through the center support of the cage. Assembly to this point was done on shore. When the site of incubation was reached, the whole assembly was lowered into the water, and a rope, previously anchored to the lake bottom was threaded through the center of the PVC pipe (DeNoyelles and O'Brien 1974). The vessels were then filled, the experimental material added, and the raft was positioned over the PVC pipe. At this time, with the vessels partially submerged, external filters (54 μm mesh) were screwed on to the entry valves, which were then opened to the lake. Two 4.5 kg weights were clipped on to the assembly sinking it to the desired depth as determined by a bolt inserted through a hole drilled at right angles through the PVC pipe. The anchor rope was then tightened and clamped. In this way, the raft was positioned directly above the experimental vessels. Secured to the raft were the pumps, toxicant reservoir, stirrer checker and battery power supply. The only maintenance required was the refilling of the toxicant reservoir and exchange of the external 54 μm filters, which can become clogged due to growth of periphyton. All experiments were incubated for 2 wk, and during this period one filter change and one refilling of the toxicant reservoir were required.

Collection of Samples

Samples for chemical analysis were taken every 3–4 d from the effluent lines to check for proper levels of toxicant in the chambers. To obtain zooplankton samples, lake water from the carboy or from the vessels after incubation was poured through a 2-stage filter. The water flowed first through a 54 μm and then a 30 μm aperture nylon mesh filter (Nitex). The zooplankton caught on each filter were combined to form a single sample and preserved in 4% formalin (final concentration). This two stage filtering system helped prevent clogging which occurred when only the 30 μm filter was used. The double system ensured that small zooplankters were not lost. Filtering was done after the vessels had been brought to shore.

Enumeration of Samples

In the laboratory, the zooplankton samples were made up to 40 mL (final) volume by filtration through a 20 μm mesh filter. Crustaceans were counted in 5 mL subsamples placed in a custom-made 75 × 75 × 10 mm counting cell. Rotifers were counted in two 1 mL aliquots in a Sedgwick–Rafter counting cell. The counts were made at 6.3× on a Zeiss binocular microscope.

To confirm effects such as species shifts or change in life cycle, an accurate method of estimating size of various populations was required. When the contents of the vessels, originally suspended in 40.75 L of water, were reduced to 40 mL, a very heterogeneous slurry resulted. Subsamples of 1, 5, and 10 mL were counted and compared. The 1 mL subsamples proved to be highly variable and an inordinate number of subsamples had to be counted for accurate estimates to be obtained. Ten mL aliquots provided more accurate estimates, but settled in several layers in the counting chamber, so that the counting procedure was very complicated and time-consuming. The method finally chosen was based on counting half the sample in 5 mL aliquots. Even numbered subsamples were drawn and put aside. The odd numbered subsamples were counted. The accuracy of estimation using this method was verified by counting all organisms in the samples of two experiments in 5 mL subsamples. Figure 3 shows animal density in four 5 mL subsamples plotted against the percentage error of this estimate, when compared to counts in eight 5 mL subsamples. Counting half the total sample provided acceptable accuracy (Woelkerling et al. 1976). The error caused by counting six 1 mL aliquots is illustrated (Fig. 3).

Typically the highest variance in population estimates occurred at low animal densities (Fig. 3). The error in estimation placed a lower limit to the sensitivity of the method. A mean error of 5 + 4.1% was realized with a minimum raw number of 70 animals. This corresponded to actual concentrations of approximately 1.75 animals$\cdot$L^{-1} (Fig. 3).

Data Analysis

Numbers of various populations in treated and control vessels were analyzed by standard statistical methods. The

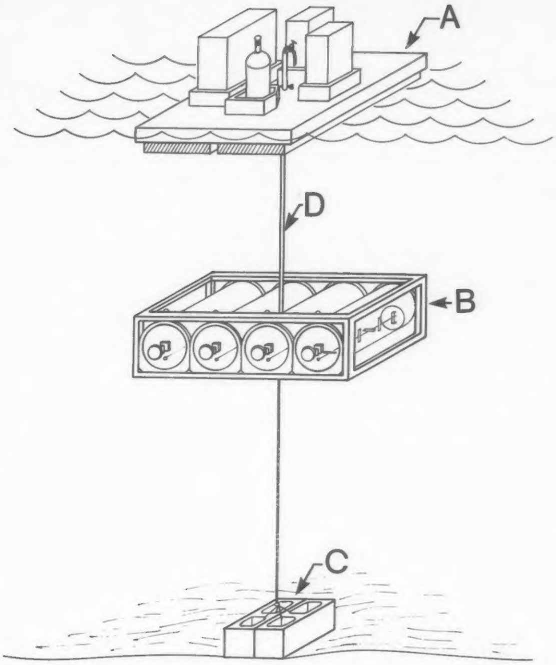


FIG. 2. Diagram of typical in situ incubation of the complete apparatus. Anchor (C) secures the raft (A) directly above itself. A PVC pipe (D) holds the cage (B) with four culture boxes at 2 m depth. The raft (A) contains all pumps, batteries, toxicant reservoir, and other apparatus necessary for operation.

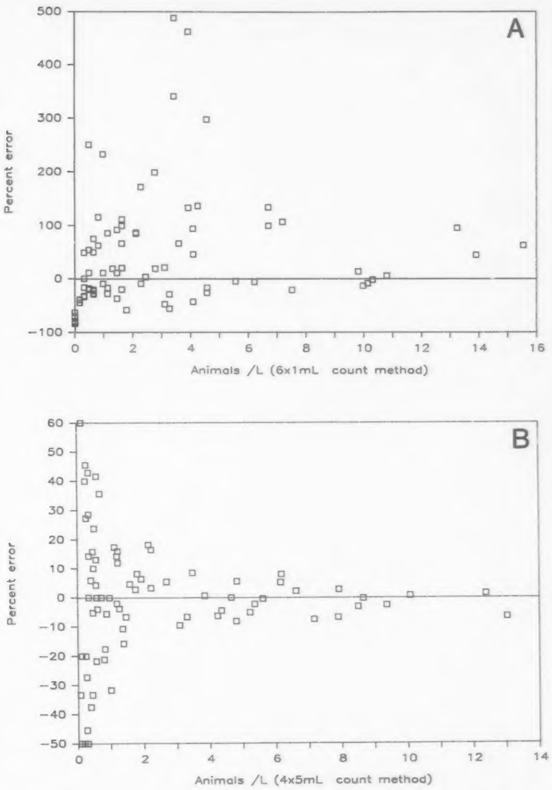


FIG. 3. Percentage errors in the estimates of total population at different animal densities. A, estimates based on counts of six 1 mL aliquots and B, four 5 mL aliquots.

method for obtaining standard error and confidence intervals for such populations is taken from Prepas (1984). To look at community structure changes, percent similarity indices based on total density (PS_1) (Bray and Curtis 1957) and species proportionality (PS_2) as used by Nicholls (1985) were calculated. As well, we used an index proposed by Nicholls (1985) based on a combination of species density and proportionality, each parameter weighted by the user (PS_3). Individual naupliar and copepodite stages were treated separately in all formulae used for calculation of percent similarity. These formulae may be calculated as:

$$PS_1 = \Sigma_i \min (x_i, y_i)$$

$$PS_2 = 100 - \frac{100}{\Sigma_i z_i} \Sigma_i x_i - \Sigma_i y_i$$

$$PS_3 = 1/2\,[\Sigma \min(x_i, y_i)] + 1/2\; 100 - \frac{100}{\Sigma_i z_i} \Sigma_i x_i - \Sigma_i y_i$$

where $\min(x_i, y_i)$ is the lesser percentage (doubly standardized) of a species in two samples X and Y and where $\Sigma_i z_i$, $\Sigma_i y_i$ are the total quantities of all species in samples Z, X, and Y, respectively. Sample Z contains the highest density of all species in the set $\Sigma_i z_i > (\Sigma_i x_i, \Sigma_i y_i)$.

Analysis of the Utility of the System

Experimental Parameters

The impoundment system was used in experiments to assess the effects of various toxic materials to the zooplankton community of Lake 302S, located in the Experimental Lakes Area (ELA) of northwestern Ontario (Brunskill and Schindler 1971). To determine the sensitivity and reproducibility of the results obtained with the apparatus, we analyzed four of these experiments. Experiments A and B were run using 5 $\mu g \cdot L^{-1}$ Zn and 0.1 $\mu g \cdot L^{-1}$ Cd, respectively as the toxicants. The metals at these concentrations were not previously shown to cause any response. In experiments C and D, 3 $\mu g \cdot L^{-1}$ Cd, shown earlier to perturb zooplankton communities in static systems (Marshall and Mellinger 1980), was maintained in the treated vessels. Though the concentrations in experiments C and D were identical, the distribution of life cycle stages were not, so in effect they constituted different experiments. All were run at a flow rate of 0.6 exchange$\cdot d^{-1}$ for two weeks. This rapid flow rate was chosen to ensure adequate food supply and metabolite removal for the entrained organisms. These experiments were performed serially from spring to late summer, so that animal diversity and population size varied in each experiment. Identical treatments would not necessarily yield exactly the same statistical similarity index values because of different starting conditions (Hill 1973). Effects on the communities, if any, would also be a function of lake chemistry and physical factors present at the time of the experimental treatment.

TABLE 1. Calanoids. Percent similarity values are presented for experiments A to D. In each experiment the mean of dissimilar crosses should be compared with the similar crosses (treated 1 vs treated 2, and control 1 vs control 2). These calculations have been performed on raw untransformed data on the 11 life cycle stages and 2 adult species, obtained after 2-wk incubation.

Experiment		PS_1	PS_2	PS_3
A. 5 $\mu g \cdot L^{-1}$ Zn	control 1 vs control 2	86.6	90.3	88.5
	treated 1 vs treated 2	84.5	95.1	89.8
	mean of dissimilar crosses	87.6	94.6	91.4
B. 0.1 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	88.9	99.1	94.0
	treated 1 vs treated 2	90.9	89.1	90.0
	mean of dissimilar crosses	89.3	94.6	91.9
C. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	48.2	52.4	50.3
	treated 1 vs treated 2	40.6	77.8	59.2
	mean of dissimilar crosses	48.8	50.8	49.8
D. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	67.6	65.5	66.6
	treated 1 vs treated 2	50.6	96.8	73.7
	mean of dissimilar crosses	47.6	29.7	38.6

TABLE 2. Cyclopoids. Percent similarity values are presented for experiments A to D. In each experiment the mean of dissimilar crosses should be compared with the similar crosses (treated 1 vs treated 2, and control 1 vs control 2). These calculations have been performed on raw untransformed data on the 11 life cycle stages and 3 adult species, obtained after 2-wk incubation.

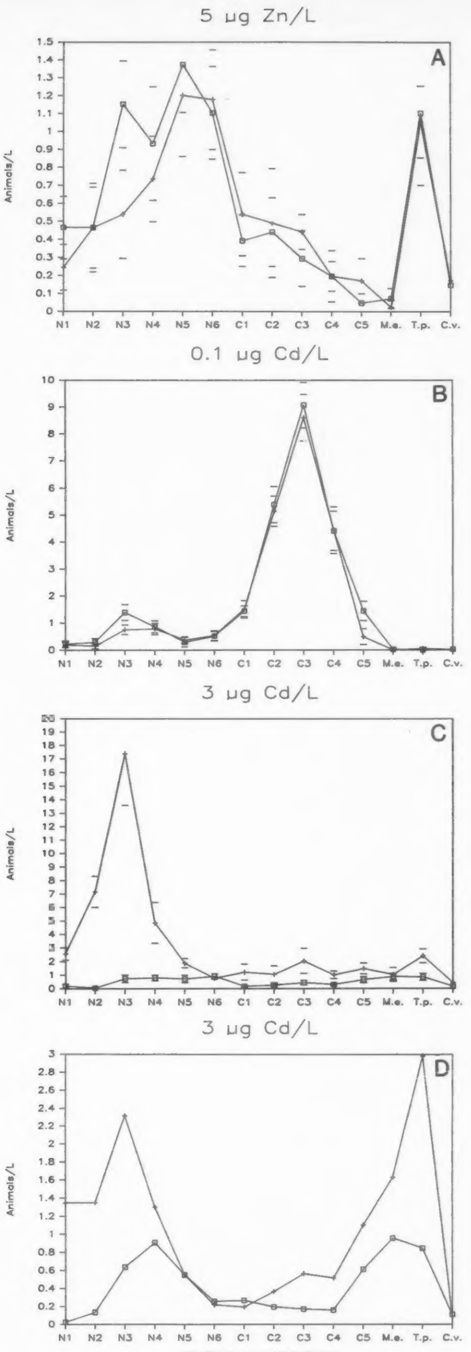
Experiment		PS_1	PS_2	PS_3
A. 5 $\mu g \cdot L^{-1}$ Zn	control 1 vs control 2	80.3	92.9	86.6
	treated 1 vs treated 2	77.0	83.5	80.3
	mean of dissimilar crosses	76.6	89.8	83.2
B. 0.1 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	80.7	95.1	87.9
	treated 1 vs treated 2	80.6	93.8	87.2
	mean of dissimilar crosses	80.5	91.1	85.8
C. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	80.2	58.6	69.4
	treated 1 vs treated 2	82.8	95.3	89.1
	mean of dissimilar crosses	64.0	33.1	48.6
D. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	82.1	87.1	84.6
	treated 1 vs treated 2	81.7	91.4	86.5
	mean of dissimilar crosses	69.8	44.2	57.0

TABLE 3. Cladocerans. Percent similarity values are presented for experiments A to D. They were calculated using data on the three or four (when present) species of cladocerans after 2-wk incubation.

Experiment		PS_1	PS_2	PS_3
A. 5 $\mu g \cdot L^{-1}$ Zn	control 1 vs control 2	69.0	85.0	77.0
	treated 1 vs treated 2	69.0	55.0	62.0
	mean of dissimilar crosses	67.6	73.4	69.6
B. 0.1 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	66.7	79.8	73.2
	treated 1 vs treated 2	58.8	75.8	67.3
	mean of dissimilar crosses	66.3	83.4	74.8
C. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	58.5	88.0	73.2
	treated 1 vs treated 2	98.0	99.0	98.6
	mean of dissimilar crosses	40.0	7.4	23.7
D. 3 $\mu g \cdot L^{-1}$ Cd	control 1 vs control 2	72.2	89.3	80.7
	treated 1 vs treated 2	81.4	97.6	89.5
	mean of dissimilar crosses	42.3	8.5	25.5

Experimental Results

Three community groups, the calanoid copepods, the cyclopoid copepods, and the cladocerans were analyzed and compared separately. Each life phase of the copepods was treated as a separate population. Sensitivity peculiar to any particular species or life phase would become evident (Tables 1, 2, and 3).



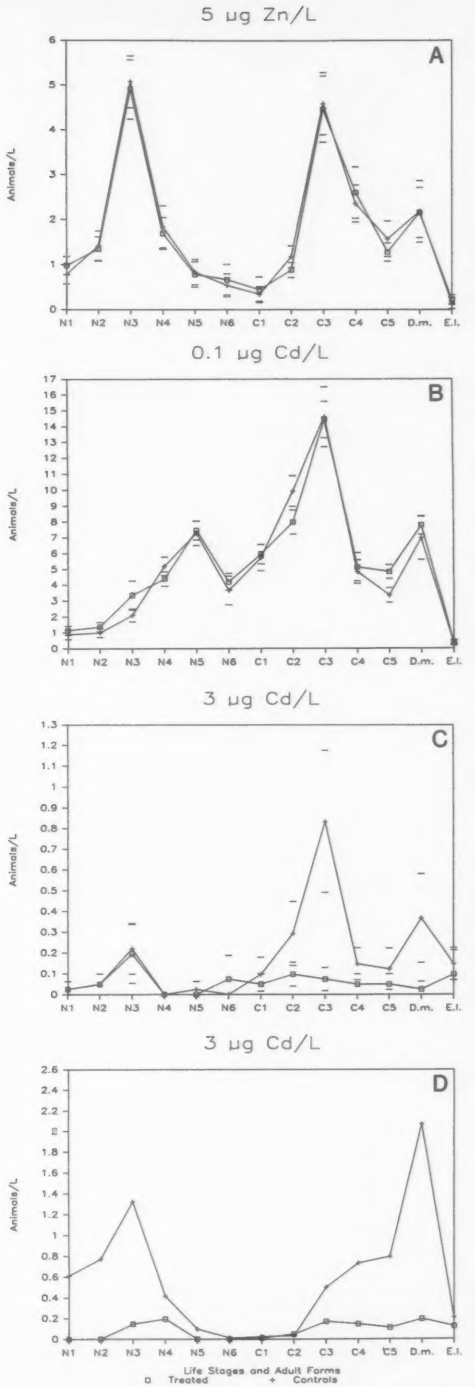
5 µg Zn/L

0.1 µg Cd/L

3 µg Cd/L

3 µg Cd/L

FIG. 4. Cyclopoid densities for experiments A to D. Controls refers to the mean of combined results of both control vessels. Similarly, treated is the mean of combined counts of the treated vessels. Naupliar and copepodite stages are followed by densities of adult *Mesocyclops edax* (M.e.), *Tropocyclops prasinus mexicanus* (T.p.), and *Cyclops vernalis* (C.v.). Bars indicate 95% confidence limits.



5 µg Zn/L

0.1 µg Cd/L

3 µg Cd/L

3 µg Cd/L

FIG. 5. Calanoid densities for experiments A to D. Controls are the mean of combined controls. Treated is the mean of both treated vessels. Naupliar and copepodite stages are followed by adult forms of *Diaptomus minutus* (D.m.) and *Epischura lacustris* (E.l.). Bars indicate 95% confidence limits.

TABLE 4. Comparison of the abundance of cladoceran species in Experiments A to D. Lake time 0 refers to the abundance present at the time of entrapment. The values and standard error for the control and treated vessels were calculated on the counts taken after the 2-wk incubation.

	Experiment	Lake time 0 Mean ± SE	Control Mean ± SE	Treated Mean ± SE
A.	$5\ \mu g\cdot L^{-1}$ Zn			
	Bosmina longirostris	2.06 (0.46)	0.96 (0.22)	1.20 (0.20)
	Diaphanosoma birgei	0.10 (0.06)	1.15 (0.15)	6.01 (1.47)
	Holopedium gibberum	1.33 (1.40)	8.86 (0.88)	9.40 (0.47)
	Daphnia galeata mendotae	0.05 (0.05)	1.03 (0.24)	0.64 (0.23)
B.	$0.1\ \mu g\cdot L^{-1}$ Cd			
	Bosmina longirostris	5.84 (0.94)	15.8 (0.97)	14.09 (1.07)
	Diaphanosoma birgei	0.15 (0.09)	0.05 (0.05)	0.07 (0.04)
	Holopedium gibberum	0.64 (0.12)	1.29 (1.84)	1.33 (0.21)
	Daphnia galeata mendotae			
C.	$3\ \mu g\cdot L^{-1}$ Cd			
	Bosmina longirostris	2.45	31.7 (2.71)	0.32 (0.07)
	Diaphanosoma birgei	0.44 (0.19)	0.59 (0.22)	0.17 (0.06)
	Holopedium gibberum		0.07 (0.05)	
	Daphnia galeata mendotae			
D.	$3\ \mu g\cdot L^{-1}$ Cd			
	Bosmina longirostris	11.39 (3.54)	11.6 (13.6)	3.36 (0.79)
	Diaphanosoma birgei	4.17 (1.28)	2.82 (0.29)	0.88 (0.20)
	Holopedium gibberum	1.18 (0.31)	6.94 (1.09)	0.07 (0.04)
	Daphnia galeata mendotae			

Experiment A

Two treated vessels were dosed with $5\ \mu g\cdot L^{-1}$ Zn (actual measurement 5.08 ± 0.73). Profiles of organisms in treated vessels showed very little difference from those in the control vessels after a 2-wk incubation (Fig. 4 and 5 and Table 4). The cyclopoid copepods showed more variability due to low animal density. The only significant difference in this experiment was in *Diaphanosoma* numbers which increased in the treated vessels (Table 4). This could be due to differences in competition or to stimulation of this species by the small amounts of added zinc.

Experiment B

A nominal $0.1\ \mu g\cdot L^{-1}$ Cd (actual measurement <0.1) was added to the treated vessels. Few differences between treated and untreated vessels can be shown. The life cycle profiles of both cyclopoid and calanoid copepods matched very closely. Cladoceran numbers (Table 4) were more variable, though the means were close. This was reflected in the reduction in the percent similarity index (Table 3). The low number of species in the cladocerans generally lowered their percent similarity values when compared to the similar statistical treatment given to the cyclopoids and calanoids and their life cycle stages.

Experiment C

A concentration of $3\ \mu g\cdot L^{-1}$ Cd (nominal) (3.55 ± 0.83 measured) was maintained in the treated vessels throughout the 2-wk experimental period. This experiment was expected to show community perturbations based on previous experience with the static bioassay system (Marshall and Mellinger 1980). A large significant ($P < 0.05$) depression in the number of nauplii (N1−4) of cyclopoid copepods and of *Tropocyclops* occurred (Fig. 4). This was also reflected in the percent similarity tables as a drop in value of the treated vs control pairings. At this time the calanoid population was very low, so though changes in life cycle stages can be shown graphically, the low density resulted in uncertain percent similarity values, regardless of pairings. Perhaps the most dramatic effect was on the cladoceran *Bosmina longirostis*, whose numbers showed two orders of magnitude decrease in the treated vessels. This decrease was reflected commensurately by the dissimilarity shown in the treated vs control pairings in Table 3.

Experiment D

Cadmium was added and maintained as in Experiment C, measured concentration 2.8 ± 0.34. Calanoid nauplii and adult *Diaptomus minutus* populations were affected by this concentration (Fig. 5). The densities of these populations were generally marginal; however, since this experiment was part of the whole vs half count trial and all eight 5 mL aliquots were counted, differences in density were in terms of absolute numbers. N1−4 cyclopoid copepods and adult *Tropocyclops* were also reduced in number. The differences in the calanoid and cyclopoid copepods are reflected in an approximate 25% decrease in similarity in treated vs control pairings (Table 3). As in Experiment C, *Bosmina* was greatly affected and, as well, *Holopedium gibberum* was shown to be very sensitive to cadmium (Table 4). The changes in these two species reduced the percent similarity values by 50% in the treated vs control pairings.

Discussion

The Impoundment System

The system described here was designed to furnish in situ conditions for impounded zooplankton communities, and to facilitate the study of experimental perturbations on them.

Large limnocorral systems and small statically incubated containers have also been used in the study of effects of toxic materials on zooplankton (Lawrence and Holoka 1983; Malley et al. 1987).

Experiments using limnocorrals are designed to investigate long-term effects on impounded populations, so the number of individual experiments which can be performed in a single season is limited, usually to one. If more than one experiment is performed in a limnocorral, the perturbations of the previous experiment may persist to confound the subsequent experimental results (Lawrence 1980). An advantage of smaller systems is that repeated short-term experiments can be performed as necessary. We have used the flow-through system for as many as nine 2-wk experiments serially. Each experiment can be treated as an entity or as part of the series. The materials used to construct our system are easily cleaned. Chemical analysis has shown that heavy metals are not retained on the walls of the vessels from one experiment to another.

Control and maintenance of entrained nutrient or toxicant levels is difficult in limnocorrals and is often not attempted because usually the response of impounded biota is being examined in the presence of decaying concentrations of added materials. Exchange of dissolved material with the outer environment is slow. In small statically incubated systems, the contents of the vessels are completely separated from the natural habitat during the experimental period. Such small systems are subject to the same variable regime as the lake only with respect to light and temperature. No exchange of nutrients, gases or metabolites can occur. The reaction of organisms to progressively increasing concentration in wastes and to the decay in nutrient sources is an effect of impoundment alone. Responses to added materials may be either masked or exaggerated because of these conditions. Exchange is an inherent feature of the flow-through system which provides for the constant renewal of expelled liquids.

Impoundment effects become more of a problem with the increasing size and decreasing natural density of organisms, e.g., for zooplankters compared to phytoplankton or bacteria. The size and shape of the vessels in the flow-through system as well as rates of exchange and stirring speed, have been chosen to accommodate the large sizes and low natural density of the zooplankton community. In contrast to the operation of the system designed for examination of phytoplankton by DeNoyelles et al. (1980), internal and external filters are used to impound the zooplankton initially placed in the vessels. This was done so that actual progressions of organisms through a life cycle could be observed without a dilution effect. Possible divergence from the lake community is compensated for by increased experimental sensitivity.

Limnocorrals provide abundant biomass, but due to their large size, they are subject to discontinuous distribution of zooplankters. This patchiness requires that a prior study be made of the distribution of impounded organisms (Stephenson et al. 1984). Only in this way can representative samples be collected during experimental periods. The flow-through apparatus eliminates this requirement. Because the entire contents of vessels can be sampled, horizontal, vertical, or temporal distribution of organisms is not an issue.

Analysis of Data

Most significant differences between organisms from treated and control vessels occurred above the level of 1.75 animals$\cdot$L^{-1}. Below this concentration, errors in population estimates of up to 60% were possible. Unless differences in population were drastic, confidence in interpretation of toxic effects on populations below this density was limited.

As indicated visually, the standard error bars for combined control and experimental vessels (Fig. 4 and 5) demonstrate low variance between matching vessels. The use of the percent similarity index is that it helps summarize consistency within matching control and treated vessels while at the same time providing a measure of similarity between treated and untreated populations. The percent similarity value may not be an absolute measure but is useful in comparing subsequent experimental results. Ideally, the similar crosses (control vs control, treated vs treated) should have a value as high as possible at all times. If an actual effect was taking place in the treated vessels, the mean of the four dissimilar crosses (treated 1 vs control 1, treated 1 vs control 2, treated 2 vs control 1, and treated 2 vs control 2) should exhibit a decreased value in comparison to the two similar crosses. Any inconsistency between graphically presented data and percent similarity value could be attributed to variability between matching pairs. This situation is illustrated in experiment C by the calanoid population. The graphed results indicate significant differences exist at later life stages. However, the percent similarity table for this experiment (Table 1) indicates values of about 50% similarity for all possible crosses. The low animal density, resulting in higher percent variability between vessels accounts for this result. If no real effect was present, results presented graphically would show similar profiles, and the percent similarity tables would show high values for all similar and mean dissimilar crosses, as happened in Experiments A and B where no effect was evident. The results of Experiments C and D were also consistent, as the graphed results and the percent similarity tables supported each other. The decreases shown in the graphs were paralleled by the reduction in the values of the mean dissimilar crosses.

The formula (PS_3) (Nicholls 1985) provided a useful summary of effects because both bases of calculation of percent similarity were treated simultaneously. However, the other formulae were included to provide information on the nature of the differences, whether based on density or species proportionality. In all cases where animal density was low (<1.75 animals$\cdot$L^{-1}), the percent similarity values declined because the absolute value of the differences increased as a percentage of the whole. The results of these experiments show that this impoundment system is useful in the investigation of experimental perturbations on zooplankton communities in natural lake settings, when the density of organisms of interest exceeds approximately 1.75 individuals$\cdot$L^{-1}.

Acknowledgments

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Effects of Low Concentrations of Cadmium on the Crustacean Zooplankton Community of an Artificially Acidified Lake

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Lawrence, S. G., and M. H. Holoka. 1987. Effects of low concentrations of cadmium on the crustacean zooplankton community of an artificially acidified lake. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 163–172.

The toxic effects of cadmium to total community and individual species of zooplankton generally decreased as pH of the lake was experimentally lowered over a number of years by additions of acids. The biomass of crustacean zooplankton held in small impoundment systems suspended in the lake for fourteen days was reduced by 60–70% when pH was 6.7–6.8 and concentrations of Cd were maintained at 1 $\mu g \cdot L^{-1}$, and by 70–80% when Cd was at 3 $\mu g \cdot L^{-1}$. At a pH of 5.9, however, biomass was reduced only 20–30% in concentrations of Cd of 1 $\mu g \cdot L^{-1}$, and at pH 5.6, biomass decreased by only 20% when subjected to 3 $\mu g \cdot L^{-1}$ Cd. The cladocerans *Bosmina longirostris* and *Holopedium gibberum* were the most sensitive to cadmium. Cladocerans were more sensitive to cadmium than calanoid copepods, and both these groups were more sensitive then cyclopoid copepods. The decrease in toxicity of cadmium with increasing H^+ may be analogous to the inhibition of uptake of cadmium by calcium.

La toxicité du cadmium pour l'ensemble d'une communauté et les espèces de zooplancton a généralement baissé à mesure que le pH du lac expérimental était réduit par l'apport d'acides sur plusieurs années. La biomasse de crustacés zooplanctoniques gardés en petites cages suspendues en milieu lacustre pendant 14 jours a été réduite de 60 à 70 % quand le pH variait de 6,7 à 6,8 et que la concentration de Cd était maintenue à 1 $\mu g \cdot L^{-1}$, et de 70 à 80 % quand cette dernière atteignait 3 $\mu g \cdot L^{-1}$. Un pH de 5,9 n'a toutefois entraîné qu'une baisse de 20 à 30 % de la biomasse quand la teneur en Cd atteignait 1 $\mu g \cdot L^{-1}$; à une teneur de 3 $\mu g \cdot L^{-1}$ et un pH de 5,6, la biomasse a connu une baisse de 20 % seulement. Les cladocères *Bosmina longirostris* et *Holopedium gibberum* étaient les plus vulnérables au cadmium, et le groupe était plus sensible que les copépodes calanoïdes. Ces deux groupes étaient toutefois plus sensibles que les copépodes cyclopéens. La baisse de la toxicité du cadmium qui suit un accroissement de [H^+] peut correspondre à l'inhibition de la captation du cadmium par le calcium.

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The effects of lowered pH on crustacean zooplankton populations and communities of northeastern American lakes subject to acid precipitation have been examined in several studies (Sprules 1975; Roff and Kwiatkowski 1977; Yan and Strus 1980; Malley et al. 1982; Yan et al. 1982; Confer et al. 1983; Keller and Pitblado 1984; Schindler et al. 1985). Generally, species diversity and total crustacean biomass decrease when H^+ increases (Confer et al. 1983; Sprules 1975; Yan et al. 1982). *Diaptomus minutus*, *Bosmina longirostris*, or both become the dominant species in these lakes. Schindler et al. (1985) found that at pH 5.1 species diversity and biomass of crustacean zooplankton did not decline, but cladocerans replaced calanoid copepods as the group which dominated the biomass. In lakes subjected to acid precipitation the decrease in pH is usually associated with an increase in the concentration of such heavy metals as nickel, copper, cadmium, zinc, and lead (Dickson 1980; Yan and Strus 1980). Of these, cadmium is one of the most toxic and its effects can be easily assessed because it has no known physiological function. Worldwide annual emissions from natural sources are about 8.43×10^5 kg and from industrial activities, 7.19×10^6 kg (Moore and Ramamoorthy 1984). Precipitation removes this cadmium effectively from the atmosphere, and, for example, concentrations in Ontario rain water range from 0.01 to 50.0 $\mu g \cdot L^{-1}$ (Moore and Ramamoorthy 1984). Muhlbaier and Tisue (1981) have predicted that the concentration of cadmium in the south basin of Lake Michigan could be deleterious to sensitive zooplankters in a few decades if deposition remains at current rates.

Cadmium appears to be more toxic to plankton than to other aquatic biota (Wong et al. 1980; Marshall and Mellinger 1980). A response at the population level can be demonstrated in phytoplankton at 2–4 $\mu g \cdot L^{-1}$ Cd (deNoyelles et al. 1980). In the laboratory, Biesinger and Christensen (1972) found that 0.2 $\mu g \cdot L^{-1}$ reduced the reproductive capacity of *Daphnia magna* and Marshall (1978) showed that 4 $\mu g \cdot L^{-1}$ caused *D. galeata mendotae* populations to decline over an extended period. Marshall and Mellinger (1980) determined that 0.6–1.0 $\mu g \cdot L^{-1}$ Cd could reduce the abundance of some species of zooplankton enclosed in 10^5 L enclosures. Recently it has been reported that the toxicity of cadmium to phytoplankton decreased with decreased pH (Peterson et al. 1984;

Peterson and Healey 1985) but no such cadmium/pH relationship in zooplankton has been reported heretofore.

We report responses of the zooplankton community in one basin of an experimentally acidified (0.3–0.5 pH units per year) lake of the Canadian Shield to low, fixed concentrations of cadmium. The premanipulated pH was 6.7–6.8 (Beamish et al. 1976). Since 1982, the pH of the basin has been lowered by addition of sulfuric acid at regular intervals during the ice-free season (Cruikshank 1984).

Using a continuous-flow apparatus described by Holoka and Lawrence (1987), we conducted 16 experiments in the south basin of Lake 302 from 1981, when the pH was 6.7–6.8 to 1984, when the pH had been depressed by more than one unit. We examined the response of impounded crustacean zooplankters to approximately 1 or 3 $\mu g \cdot L^{-1}$ Cd at each pH level during the experimental acidification by measuring their dry weight biomass.

Methods and Materials

The Lake Site

Lake 302 is located at 49°40′N, 93°46′W in northwestern Ontario (Cleugh and Hauser 1971). During the experiment, two basins were completely separated by a sea curtain. These basins are designated Lake 302 south (302S) and Lake 302 north (302N). Bathymetry and chemical parameters of the lake are described by Brunskill and Schindler (1971). Lake 302 was the site of an experimental manipulation (Schindler et al. 1980) in which carbon, nitrogen and phosphorus were injected into the hypolimnion of Lake 302N annually for several years. Lake 302S served as a control. Water chemistry returned to baseline levels after termination of the experiment in 1976 (Linsey 1985).

In 1981, the lake became the site of an acidification program. Sulfuric acid was added to the south basin lowering the pH annually in increments of about 0.3–0.5 pH units, from 1982 to 1984. Nitric acid was added to the north basin. Further details of additions to Lake 302 in 1982 and 1983 are described in Cruikshank (1984). The experimental continuous-flow system was set up in the south basin of the lake (302S) only.

Samples of lake water from both basins were taken every two to three weeks during the ice-free season and monthly in the winter. These were analyzed for major ions, heavy metals, dissolved and particulate material, and suspended biota. Measurements of H^+, pH, alkalinity, and conductivity were made weekly during the ice-free season.

The Impoundment System

The flow-through impoundment system is described in detail in Holoka and Lawrence (1987). Briefly, the system was composed of four vessels (two control vessels, two treated with cadmium) each of which contained 40.75 L continuously stirred (at 16 rpm) lake water. The vessels were suspended below a raft. Four pumps drove the system. The two principal pumps pulled lake water into the vessels. Two other small volume pumps attached in tandem to the main pumps drew on a reservoir of cadmium in solution. This solution was drawn into the experimental vessels at each pumping event so that the [Cd] was maintained at the chosen level. Dilution was semicontinuous, one pumping event per min at the rate of 0.6 per day. Filters which restricted movement of zooplankton into and out of vessels were 54 μm nylon (Nitex). Incubation depth was 2 m; incubation time was 14 d.

Sampling and Enumeration of Samples

A Pennak sampler (Pennak 1962) was used to obtain integrated samples from the surface of the water to 4 m. These samples were placed into a 75 L plastic container from which the vessels were simultaneously filled. At the beginning of an experiment, 45.8 L lake water sample was placed in a carboy as the vessels were being filled, using the mixing and dividing system as described by Holoka and Lawrence (1987). This provided a time$_0$ sample of zooplankton which was immediately filtered as described below. After the 14 d incubation, the contents of each of the vessels were filtered sequentially through 54 μm and 30 μm nylon filters to prevent clogging of the smaller mesh filter; zooplankters >30 μm in the smallest dimension were retained for enumeration. Samples were preserved in 4% (final concentration) formalin.

Identifications of copepods were made according to Wilson and Yeatman (1959) and of cladocerans according to Brooks (1959). Crustaceans were counted as described in Holoka and Lawrence (1987), i.e., the zooplankton in 50%, occasionally 100%, of each sample were identified and counted in four 5 mL aliquots. Nauplii (N1–6) and immature copepodids (C1–5) were identified to life stage, and designated as either calanoid or cyclopoid copepods. Mature copepods were identified to species and to sex. Copepodids 3 to 5 and mature (adult) copepods were assigned to a copepod species and treated as a group, C3-adult, for that species. Copepodids 3–5 in a given sample were apportioned among the species according to the abundance of adults of each species in that sample.

Copepodid 3-adult forms of *Epischuria lacustris*, *Mesocyclops edax*, *Cyclops vernalis* and *C. bicuspidatus thomasi* were designated as predaceous organisms (Williamson 1980, 1984; Kerfoot 1977, 1978; Wong 1981). Cyclopoid and calanoid nauplii and copepodids 1–2, C3-adult *D. minutus*, C3-adult *Tropocyclops prasinus mexicanus*, and the cladocerans *B. longirostris*, *Diaphanosoma birgei* and *Holopedium gibberum* were designated as herbivorous or filtering organisms.

Estimates of the dry weight biomass of individual life stages of the six copepod and three cladoceran species were made by the methods of Lawrence et al. (1987). The dry weight estimates for copepods and the length–weight regressions calculated for cladocerans appear in Table 1. The length–weight regressions listed for 1981 were used to estimate biomass in experiments of 1981 and 1982. Those listed for 1983 were used to generate estimates for 1983 and 1984.

Cadmium concentrations were measured using a Varian AA-5 atomic absorption spectrophotometer. From 1981 to 1983 samples were measured without manipulation. In 1984, nitric acid was added to the sample prior to assessment.

Statistical Analysis

The basic statistical treatment of data used to test if treated biomass differed from control was the nested analysis of variance (ANOVA), vessels nested within treatments at $\alpha = 0.05$. When data were available from fewer than four vessels, the standard one-way ANOVA was used. Because 50% of each sample was enumerated, a finite population correction was applied to the standard error of the mean (Prepas 1984).

TABLE 1. (A) Calculated dry weight estimates. Values for life phases of copepods are calculated from mean dimensions (Lawrence et al. 1987). N is the number of individuals measured. Values are means ± SE. (B) Length−weight regressions of cladocerans are generated from measured lengths and calculated biomass as in Lawrence et al. (1987). The number of observations (N), intercept ($\ln a$), the slope (b), 95% confidence levels (C.I.), the average logarithm of the length in mm ($\bar{x}$), estimated variance of $\bar{x}$, ($s^2_{\bar{x}}$), residual mean square (RMS), correlation coefficient (r), and the range of length measurements are presented for the relationship $\ln w = \ln a \pm b\bar{x}$, where $\ln w$ is the logarithm of the dry weight (μg).

A. Estimated dry weights for copepods

Life phase	N	μg·individual^{-1}	Formula	Life phase	N	μg·individual^{-1}	Formula
Calanoid copepods				Cyclopoid copepods			
N1	20	0.038 ± 0.002	$4/3\pi ab1.5c$	N1	20	0.011 ± 0.0008	$4/3\pi ab1.5c$
N2	20	0.049 ± 0.002	″	N2	20	0.018 ± 0.002	″
N3	20	0.075 ± 0.002	″	N3	27	0.023 ± 0.002	″
N4	20	0.110 ± 0.004	″	N4	22	0.036 ± 0.003	″
N5	20	0.143 ± 0.004	$4/3\pi ab1.4c$	N5	20	0.062 ± 0.003	$4/3\pi ab1.4c$
N6	20	0.144 ± 0.006	$4/3\pi ab1.25c$	N6	19	0.100 ± 0.005	$4/3\pi ab1.25c$
C1	20	0.245 ± 0.016	″	C1	21	0.145 ± 0.024	″
C2	20	0.470 ± 0.014	″	C2	19	0.230 ± 0.038	″
D. minutus				*T. p. mexicanus*			
C3	20	0.779 ± 0.024	″	C3	16	0.134 ± 0.015	″
C4	20	1.234 ± 0.036	″	C4	18	0.233 ± 0.012	″
C5	20	1.740 ± 0.081	″	C5	18	0.357 ± 0.028	″
Male	61	1.962 ± 0.054	″	Male	46	0.229 ± 0.006	″
Female	86	2.571 ± 0.073	″	Female	55	0.458 ± 0.012	″
E. lacustris				*M. edax*			
C3	—	3.495[a]	″	C3	8	0.950 ± 0.071	$4/3\pi ab1.5c$
C4	—	5.536[a]	″	C4	10	1.743 ± 0.115	″
C5	—	7.807[a]	″	C5	14	4.126 ± 0.399	″
Male	16	9.140 ± 0.354	″	Male	32	1.690 ± 0.047	″
Female	9	11.535 ± 0.486	″	Female	30	5.872 ± 0.225	″
				C. vernalis			
				C3	9	0.611 ± 0.049	$4/3\pi abc$
				C4	16	1.253 ± 0.092	″
				C5	11	1.321 ± 0.098	″
				Male	39	1.472 ± 0.042	″
				Female	39	5.359 ± 0.243	″
				C. b. thomasi			
				C3	—	0.244[b]	″
				C4	—	0.326[b]	″
				C5	—	0.611[b]	″
				Male	10	0.581 ± 0.024	″
				Female	28	0.872 ± 0.039	″

B. Length–weight regressions for cladocerans

Species	Year	N	$\ln a$	b ± 95% C.I.	$\bar{x}$	$s^2_{\bar{x}}$	RMS	r	Range length (mm)	Formula
B. longirostis	1981	100	1.8747	2.9074 ± 0.1626	−1.2140	0.0470	0.0314	0.96	0.184−0.432	$4/3\pi abc$
	1983	78	1.7930	2.7151 ± 0.2539	−1.2543	0.0793	0.1004	0.92	0.112−0.520	″
D. birgei	1981	50	1.0436	2.7318 ± 0.2111	−0.3224	0.1235	0.0674	0.97	0.432−1.760	″
	1983	50	0.9740	2.6410 ± 0.1935	−0.3452	0.0582	0.0267	0.97	0.404−1.096	″
H. gibberum	1981	50	2.2852	2.4328 ± 0.3646	−0.5098	0.0699	0.1138	0.89	0.352−1.028	$4/3\pi ab3c<1000$ μm, $1.5c>1000$ μm
	1983	52	2.4285	2.5195 ± 0.3376	−0.4477	0.0804	0.1168	0.90	0.320−1.128	″

[a] Value estimated. Ratio of value for *D. minutus* C3 (4, 5) to value for *D. minutus* female was multiplied by value for *E. lacustris* female to obtain values for *E. lacustris* C3-5.

[b] Value estimated. Ratio of values for Lake 223 *C. b. thomasi* C3 (4, 5) to value for Lake 223 *C. b. thomasi* female (Lawrence et al. 1987) was multiplied by value for Lake 302 *C. b. thomasi* female to obtain values for Lake 302 *C. b. thomasi* C3-5.

TABLE 2. Physical and chemical conditions in Lake 302S during experiments. Values of pH and dissolved organic carbon (DOC) (after 3 August 1982) are means. pH, T°C and DOC values for 1981 to 1983 from Linsey et al. (1985); 1984 values from D.W. Schindler, Freshwater Institute, unpublished data. SE is the standard error, (*n*) indicates number of measurements.

	Treatment of vessels ($\mu g\ Cd \cdot L^{-1}$)				
Year	Nominal concentration	Measured mean ± SE (*n*)	pH[a]	T°C (epilimnion)	DOC $\mu g \cdot L^{-1}$
1981					
May 28–June 11	3	2.34±0.1 (12)	6.80	17.2 (June 9)	415 (June 9)
July 9–22	3	2.61±0.2 (8)	6.76	22.2 (July 7)	440 (July 7)
July 28–Aug. 11	1	1.03±0.1 (7)	6.74	22.7 (Aug 4)	425 (Aug 4)
Aug. 25–Sept. 9	1	0.98±0.2 (4)	6.77	20.9 (Sept 1)	555 (Sept 1)
1982					
May 26–June 5	1	1.32±0.3 (6)	6.63	11.2 (May 18)	1220 (June 1)
July 20–Aug. 4	1	1.30±0.3 (6)	6.35	16.4 (June 15)	850 (Aug 3)
Aug. 4–17	3	2.7	6.34	20.2 (Aug 10)	900
Aug. 17–Sept. 1	3	3.06±0.2 (7)	6.34	16.3 (Sept 7)	620
1983					
June 28–July 12	1	0.83±0.05 (6)	5.84	20.5 (July 12)	390
July 13–26	1	0.80±0.12 (8)	5.84	20.5 (July 12)	390
July 27–Aug. 9	3	2.07±0.1 (3)	5.84	24.1 (Aug 9)	390
Aug. 10–23	3	3.08±0.4 (8)	5.87	24.1 (Aug 9)	370
Aug. 24–Sept. 6	3	2.30±0.05 (3)	5.85	21.1 (Sept 6)	365
Sept. 6–20	1	1.68±0.2 (4)	5.99	21.1 (Sept 6)	390
1984					
May 23–June 5	3	3.97±0.5 (5)	5.63	13.9 (May 29)	415
June 6–19	3	3.57±0.2 (3)	5.53	—	330

[a] Lake acidification program started 29 June 1982.

Results

Cadmium Concentrations in Experimental Vessels

The nominal concentrations of 1 or 3 $\mu g \cdot L^{-1}$ Cd were maintained reasonably well in treated vessels (Table 2). The values for 1984 are higher than in preceding years, probably because the handling of samples was altered in that year.

Chemistry of Lake 302 South

The pH of L302S was depressed by 0.5 pH unit in 1982. In 1983 and 1984, the pH decreased 0.3 units per year (Table 2). Alkalinity decreased from 50 $\mu eq \cdot L^{-1}$ in 1981 to 4.5 $\mu eq \cdot L^{-1}$ in 1984 (Linsey et al. 1985; M. Turner, Freshwater Institute, personal comm.). Dissolved organic carbon (DOC) values were three times higher in 1982 than in other years (Table 2). Lake temperatures were generally lower in 1982 than in 1981 or 1983 (Table 2).

The Zooplankton Community

The principal crustacean zooplankton of the lake are (1) the calanoid copepods *D. minutus*, and *E. lacustris*; (2) the cyclopoid copepods *T. p. mexicanus*, *M. edax*, and *C. vernalis*; and (3) the cladocerans *B. longirostris* and *H. gibberum* (Ramsey 1985). The cyclopoid *C. b. thomasi* and the cladoceran *D. birgei* appeared only occasionally, but sometimes in large enough numbers that their biomass was included in calculations. Other organisms seen occasionally in low numbers were the cladocerans *Leptodora kindti*, *Chydorus sphaericus*, *Ophryonus gracilis*, *Alona* sp., and a *Daphnia* sp. The biomass of these organisms was not included in calculations.

TABLE 3. Ratios of biomass in vessels at the end of experimental periods: biomass at the beginning.

Year	Lake pH	3 $\mu g \cdot L^{-1}$	Control	1 $\mu g \cdot L^{-1}$	Control
1981	6.7–6.8	0.6	1.9	1.8	4.3
		0.5	2.9	3.0	10.5
1982	6.3–6.6	0.9	2.3	2.8	2.9
		0.4	1.7	1.3	1.6
1983	5.8–5.9	0.9	2.4	3.0	2.6
		0.4	1.1	3.1	3.9
		0.5	1.7		
1984	5.5–5.6	4.0	4.2		
		1.9	2.4		

Zooplankton Biomass

The biomass in control vessels increased from time$_o$ by as much as 10.5 times (Table 3). The biomass of vessels treated with 3 $\mu g \cdot L^{-1}$ Cd decreased from the time$_o$ level except in 1984. The biomass in vessels treated with 1 $\mu g \cdot L^{-1}$ Cd increased over the time$_o$ biomass, but only by about half as much as biomass increases in control vessels.

In 1981 when the lake pH was 6.7–6.8, the biomass of impounded communities was reduced by 70–80% with exposure to 3 $\mu g \cdot L^{-1}$ Cd (Table 4). In each succeeding year, as the pH of the lake was lowered, the relative reduction in

TABLE 4. Biomass in μg per vessel of the crustacean zooplankton communities in control and treated vessels after 14 d incubation. Values are means ± SE. *N* indicates number of replicates. S, NS designate significantly different, not significantly different at $\alpha = 0.05$, analysis of variance (ANOVA).

[Cd]/Year	pH	Control	*N*	Treated	*N*	Result of ANOVA
3 $\mu g \cdot L^{-1}$						
1981	6.7–6.8					
May 28–June 11		3401 ± 109	8	1056 ± 62	8	S
July 9–22		1914 ± 81	4	332 ± 20	4	S
1982	6.3					
Aug. 4–17[a]		909 ± 63	8	362 ± 33	8	S
Aug. 17–Sept. 1		1710	—	413	—	—
1983	5.8–6.0					
July 27–Aug. 9		1174 ± 43	8	449 ± 17	4	S
Aug. 10–23		2025 ± 64	4	432 ± 20	8	S
Aug. 24–Sept. 6		1298 ± 90	4	308 ± 29	4	S
1984	5.5–5.6					
May 23–June 5		3809 ± 87	8	3557 ± 64	4	NS
June 6–19		2504 ± 86	8	1869 ± 55	4	S
1 $\mu g \cdot L^{-1}$ Cd						
1981	6.7–6.8					
July 28–Aug. 11		2940 ± 107	8	1192 ± 46	8	S
Aug. 25–Sept. 9		797 ± 41	4	266 ± 49	4	S
1982	6.3–6.6					
May 26–June 8[a]		3323	—	3145	—	—
July 20–Aug. 4		1815 ± 120	4	1389 ± 56	4	NS
1983	5.8–6.0					
June 28–July 12		2580 ± 104	8	1741 ± 63	8	S
July 13–26		1127 ± 80	4	948 ± 49	8	NS
Sept. 7–20		1503 ± 224	4	1173 ± 30	8	NS

[a] All crustacea in sample counted, SE falls to 0.

biomass was relatively less with this same treatment. By 1984, when the lake pH was 5.6, crustacean biomass in treated and control vessels was either the same ($P > 0.05$) or very similar. Results of experiments conducted at 1 $\mu g \cdot L^{-1}$ Cd followed the same general pattern with one exception, May 28–June 5, 1982. This experiment was carried out prior to the initiation of acid additions to the lake (Table 2). The effects of cadmium on the crustacean community (Table 4) was predicted to be similar to those seen in 1981 with addition of this [Cd]. The lack of response was particularly marked in the Cladocera (Table 5). This group was again affected by 1 $\mu g \cdot L^{-1}$ Cd later in the summer following the initiation of acid additions to the lake. The most probable reason for this apparent anomoly was the increased concentration of organic ligands as expressed by [DOC] (Table 2), which were generally high during 1982 and reached their peak in May. The responses are not related to changes in water column temperature (Table 2) during the experimental periods.

The biomass of the calanoid copepods was significantly reduced (Table 5) by treatment with 3 $\mu g \cdot L^{-1}$ Cd at pH 6.7–5.85 (1981–83) with one exception. In the first experiment of 1982 (Aug. 4–17), *E. lacustris*, a predaceous copepod whose biomass was not significantly reduced, contributed the principal calanoid biomass in all vessels in this experiment. The biomass of calanoids was not significantly reduced by this treatment in 1984 when lake pH was 5.5–5.6. Biomass of calanoid copepods was significantly reduced only once by 1 $\mu g \cdot L^{-1}$ Cd late in 1981.

The cyclopoid copepods are variable in their sensitivity to 3 $\mu g \cdot L^{-1}$ Cd. Of the four species present in the lake, *T. p. mexicanus* and *M. edax* always occur, *C. vernalis* is usually present, and *C. b. thomasi* occurs occasionally in high numbers. In all experiments, either *M. edax* or *C. vernalis* produced the highest cyclopoid biomass in control and treated vessels. The cyclopoids as a group are somewhat less sensitive to Cd than either the calanoids or the Cladocera.

The Cladocera are more sensitive to both 1 and 3 $\mu g \cdot L^{-1}$ Cd (Table 5) than are either group of copepods. The biomass of control and treated vessels is significantly different in all but one case. These differences become less marked as the pH of the lake is lowered.

Bosmina longirostris dominated the crustacean plankton most often (Table 6) and it was a major component of the community in all experiments except for two in 1983. The decline in the biomass of *B. longirostris* in the presence of cadmium was much more marked than that of the community as a whole in 1981 and 1982 when the lake pH was above 6. The ratio of the biomass of *Bosmina* to total biomass treated with 3 $\mu g \cdot L^{-1}$ Cd ranged from 0.004 to 0.03 in 1981 and 1982, but for the community as a whole, the range of this ratio was 0.17–0.40. In 1983–84, at lake pH 5.9–5.5, the range of the ratios for *Bosmina* biomass in treated vessels was higher, 0.05 to 0.96, while that of community biomass was 0.30–0.95. In 1981–82, the average biomass formed by *Bosmina* in vessels treated with 3 $\mu g \cdot L^{-1}$ Cd was 1.2% of the total compared to 20.5% in control vessels. In 1983–84, these

TABLE 5. Biomass in μg per vessel of calanoid and cyclopoid copepods and of cladocerans in control and treated vessels after 14 d incubation. Values are means ± SE. Numbers in parentheses indicate percent of biomass represented by each group in control and treated vessels. S, NS designate significantly different, not significantly different at $\alpha = 0.05$, analysis of variance (ANOVA). Number of replicates as in Table 4.

[Cd]/Year	pH	Control	Treated	Result of ANOVA
3 $\mu g \cdot L^{-1}$Cd				
1981	6.7–6.8			
May 28–June 11				
Calanoids		2320 ± 73 (68)	627 ± 57 (59)	S
Cyclopoids		564 ± 12 (17)	426 ± 22 (40)	NS
Cladocera		516 ± 47 (15)	3 ± 0.4 (<1)	S
July 9–22				
Calanoids		597 ± 29 (31)	144 ± 14 (43)	S
Cyclopoids		548 ± 20 (29)	93 ± 13 (28)	S
Cladocera		768 ± 39 (40)	95 ± 7 (29)	S
1982	6.3			
Aug. 4–17				
Calanoids		192 ± 19 (21)	107 ± 25 (30)	NS
Cyclopoids		402 ± 48 (44)	241 ± 14 (67)	NS
Cladocera		315 ± 20 (35)	13 ± 3 (4)	S
Aug. 17–Sept. 1[a]				
Calanoids		441 (26)	117 (28)	—
Cyclopoids		454 (26)	240 (58)	—
Cladocera		814 (48)	57 (14)	—
1983	5.8–6.0			
July 27–Aug. 9				
Calanoids		156 ± 11 (13)	50 ± 11 (11)	S
Cyclopoids		587 ± 30 (50)	202 ± 29 (45)	S
Cladocera		432 ± 18 (37)	196 ± 10 (44)	S
Aug. 10–23				
Calanoids		457 ± 23 (23)	53 ± 3 (10)	S
Cyclopoids		623 ± 38 (31)	447 ± 19 (84)	S
Cladocera		944 ± 11 (47)	33 ± 3 (6)	S
Aug. 24–Sept. 6				
Calanoids		296 ± 23 (23)	95 ± 7 (31)	S
Cyclopoids		452 ± 59 (35)	188 ± 23 (61)	S
Cladocera		550 ± 35 (42)	25 ± 4 (8)	S
1984	5.5–5.6			
May 23–June 5				
Calanoids		307 ± 24 (8)	343 ± 26 (10)	NS
Cyclopoids		601 ± 39 (16)	552 ± 42 (16)	NS
Cladocera		2901 ± 32 (76)	2662 ± 16 (75)	S
June 6–19				
Calanoids		1173 ± 56 (47)	944 ± 3 (50)	NS
Cyclopoids		580 ± 46 (23)	342 ± 15 (18)	S
Cladocera		750 ± 22 (30)	583 ± 19 (31)	S
1 $\mu g \cdot L^{-1}$ Cd				
1981	6.7–6.8			
July 28–Aug. 11				
Calanoids		522 ± 42 (18)	114 ± 15 (10)	NS
Cyclopoids		1456 ± 83 (50)	957 ± 43 (80)	S
Cladocera		962 ± 28 (33)	120 ± 8 (10)	S
Aug. 25–Sept. 9				
Calanoids		93 ± 14 (12)	22 ± 8 (8)	S
Cyclopoids		431 ± 15 (54)	238 ± 39 (90)	S
Cladocera		273 ± 12 (34)	5 ± 1 (2)	S
1982	6.3–6.6			
May 28–June 8[a]				
Calanoids		1717 (52)	1839 (59)	—
Cyclopoids		656 (20)	581 (18)	—
Cladocera		949 (29)	724 (23)	—
July 20–Aug. 4				
Calanoids		1159 ± 80 (64)	1044 ± 65 (75)	NS
Cyclopoids		346 ± 30 (19)	233 ± 16 (17)	NS
Cladocera		309 ± 15 (17)	112 ± 7 (8)	S
1983	5.8–6.0			
June 28–July 12				
Calanoids		1169 ± 44 (45)	1039 ± 48 (60)	NS
Cyclopoids[b]		99 ± 11 (4)	197 ± 21 (11)	S
Cladocera		1312 ± 74 (51)	506 ± 28 (29)	S
July 13–26				
Calanoids		467 ± 30 (41)	352 ± 20 (37)	NS
Cyclopoids		225 ± 32 (20)	322 ± 36 (34)	NS
Cladocera		435 ± 28 (39)	274 ± 18 (29)	S
Sept. 7–20				
Calanoids		422 ± 34 (28)	204 ± 11 (17)	NS
Cyclopoids		463 ± 31 (31)	559 ± 39 (48)	NS
Cladocera		619 ± 94 (41)	410 ± 25 (35)	NS

[a]All crustacea in sample counted, SE falls to 0.

[b]Biomass of treated organism significantly higher than that of control organisms.

values were 26.6 and 33.8%, respectively.

In 1981–82, the average biomass formed by *Bosmina* in vessels treated with 1 $\mu g \cdot L^{-1}$ Cd and in control vessels was 7.3 and 16.8%, respectively. In 1983, these values were 5.0 and 16.3%. The biomass of *Bosmina* was prominent in only one of the three experiments in 1983, and was higher (not significantly) in treated vessels in the two experiments with low initial *Bosmina* biomass.

Holopedium gibberum is the most sensitive of the planktonic crustacea to the presence of Cd (Table 6). In 1981, at lake pH 6.7–6.8, the entire population of *Holopedium* died when treated with 3$\mu g \cdot L^{-1}$ Cd. Six percent of the biomass survived at 1 $\mu g \cdot L^{-1}$. These responses did not lessen with successive decline in pH until 1983, at lake pH 5.8 and 1 $\mu g \cdot L^{-1}$ Cd, and 1984, at lake pH 5.6 and 3 $\mu g \cdot L^{-1}$ Cd. Biomass of organisms was lower by 33–61% and 20–47% in those years, respectively.

The averaged ratios of treated to control biomass of various groups and species (Table 7) also show that the response over the experimental periods to the toxic effects of cadmium is greatest in *H. gibberum* and least in the cyclopoid copepods.

When the biomass of the crustacean community is separated according to feeding habit, that is, into a filtering or herbaceous group and a predaceous group, the filterers are found to be more susceptible to the toxic effects of cadmium than the predators (ANOVA at $\alpha = 0.05$). The principal herbivore was usually *B. longirostris* and the prominent predator was *M. edax*. At 1 and 3 $\mu g \cdot L^{-1}$ Cd the averaged ratios of treated

TABLE 6. Biomass in μg per vessel of *Bosmina longirostris* and *Holopedium gibberum* in control and treated vessels after 14 d incubation. Values are means ± SE. Numbers in parentheses indicate percent of biomass represented by each organism in control and treated vessels. S, NS designate significantly different, not significantly different at $\alpha = 0.05$, analysis of variance (ANOVA). Number of replicates as in Table 4.

[Cd]/Year	pH	Control	Treated	Result of ANOVA	[Cd]/Year	pH	Control	Treated	Result of ANOVA
3 μg·L^{-1}Cd					1 μg·L^{-1} Cd				
1981	6.7–6.8				*1981*	6.7–6.8			
May 28–June 11					July 28–Aug. 11				
B. longirostris		283 ± 18 (8)	3 ± 0.04 (>1)	S	*B. longirostris*		643 ± 28 (22)	2 ± 0.3 (<1)	S
H. gibberum		230 ± 31 (7)	0 (0)	S	*H. gibberum*		33 ± 4 (1)	2 ± 1(1)	S
July 9–22					Aug. 25–Sept. 9				
B. longirostris		330 ± 4 (17)	1 ± 0.2 (0)	S	*B. longirostris*		93 ± 3 (12)	3 ± 1 (1)	S
H. gibberum		203 ± 39 (11)	0 (0)	S	*H. gibberum*		180 ± 11 (23)	0 (0)	S
1982	6.3				*1982*	6.3–6.6			
Aug. 4–17					May 28–June 8[a]				
B. longirostris		290 ± 17 (32)	2 ± 0.4 (<1)	S	*B. longirostris*		836 (25)	717 (23)	—
H. gibberum[b]		—	—	—	*H. gibberum*		109 (3)	6 (<1)	—
Aug. 17–Sept. 6[a]					July 20–Aug. 4				
B. longirostris		442 (25)	12 (3)	—	*B. longirostris*		153 ± 10 (8)	57 ± 4 (4)	S
H. gibberum		334 (20)	6 (1)	—	*H. gibberum*[b]		—	—	—
1983	5.8–6.0				*1983*	5.8–6.0			
July 27–Aug. 9					June 28–July 12				
B. longirostris		349 ± 20 (30)	196 ± 9 (44)	S	*B. longirostris*		9 ± 1 (<1)	13 ± 1 (<1)	NS
H. gibberum[b]		—	—	—	*H. gibberum*		1262 ± 71 (49)	488 ± 28 (28)	S
Aug. 10–23					July 13–26				
B. longirostris		802 ± 7 (40)	33 ± 3 (6)	S	*B. longirostris*		253 ± 7 (45)	42 ± 3 (9)	S
H. gibberum		84 ± 4 (4)	0 (0)	S	*H. gibberum*		163 ± 22 (29)	230 ± 19 (48)	NS
Aug. 24–Sept. 6					Sept. 7–20				
B. longirostris		354 ± 22 (27)	25 ± 3 (8)	S	*B. longirostris*		47 ± 3 (3)	60 ± 4 (5)	NS
H. gibberum		193 ± 25 (15)	0 (0)	S	*H. gibberum*		568 ± 94 (38)	345 ± 21 (29)	NS
1984	5.5–5.6								
May 23–June 5									
B. longirostris		2346 ± 15 (62)	2218 ± 6 (62)	S					
H. gibberum		555 ± 33 (15)	443 ± 18 (12)	NS					
June 6–19									
B. longirostris		274 ± 14 (11)	237 ± 5 (13)	S					
H. gibberum		591 ± 9 (24)	313 ± 13 (17)	S					

[a] All crustacea in sample counted, SE falls to 0.

[b] *H. gibberum* not present in water column.

to control biomass for filterers and predators (Table 7) most closely resemble those for *Bosmina* and cyclopoid copepods.

Three unrepeated experiments in which cadmium concentrations were variable were performed in 1981 at lake pH 6.7–6.8 (Table 8). The calanoid and cyclopoid copepods exhibited variable reactions possibly because of the presence of different proportions of filtering and predaceous organisms from experiment to experiment. *Bosmina* biomass rose in treated vessels as the [Cd] fell so that at 0.7–0.2 μg·L^{-1} Cd it was nearly twice the value in control vessels. *Holopedium* remained very sensitive to the toxic effects of Cd at these low concentrations.

TABLE 7. Averaged ratios of treated to control biomass throughout experimental periods. $N = 9$ and 7 for experiments conducted at 3 and 1 μg·L^{-1} Cd, respectively, except for *H. gibberum* where $n = 7$ and 6, respectively.

Nominal [Cd]	3 μg·L^{-1}	1 μg·L^{-1}
Calanoid copepods	0.44	0.65
Cyclopoid copepods	0.56	1.05
Cladocera	0.27	0.42
B. longirostris	0.28	0.59
H. gibberum	0.19	0.42
Herbivores	0.33	0.56
Predators	0.57	1.08

Discussion

Ramsey (1985) described the crustacean zooplankton community in limnocorrals in the littoral zone of Lake 302S at pH 4.2. He cited eight of the nine prominent crustacean zooplankton in this study as "principal species" in terms of abundance. He did not see *C. vernalis*, probably because this organism prefers deeper water.

The biomass of crustacean zooplankton in lakes varies during the ice-free season. It is low in early spring and rises to a peak in late spring. During summer, the biomass decreases, usually to the ice-free season low, and then tends to rise again in early fall. The biomass of zooplankton impounded in control vessels of the flow-through system always increased over initial levels (Table 3), an effect of enclosure. Fish and large invertebrates such as *Chaoborus* are never included in the

TABLE 8. Biomass in μg per vessel of calanoid and cyclopoid copepods, *B. longirostris* and *H. gibberum* in control and treated vessels after 14-d incubation (1981) where cadmium concentrations decayed with time as indicated. Values are means ±SE. *N* indicates number of replicates. S, NS designates significantly different, not significantly different at $\alpha = 0.05$, analysis of variance (ANOVA).

Experiment duration	[Cd] $\mu g \cdot L^{-1}$	Control (A)	*N*	Treated (B)	*N*	B:A	Result of ANOVA
June 11–24	2.5–0.5		4		8		
Calanoids		1401±88		681±28		0.49	S
Cyclopoids		470±33		205±35		0.44	S
B. longirostris		114±6		3±1		0.03	S
H. gibberum		316±9		2±2		0.01	S
Aug 11–24	0.8–0.4		8		4		
Calanoids		123±16		188±20		1.53	NS
Cyclopoids		680±34		567±1		0.83	NS
B. longirostris		100±4		35±1		0.35	S
H. gibberum		398±78		0		0	S
June 25–July 8	0.7–0.2		4		8		
Calanoids		572±26		314±21		0.55	S
Cyclopoids		588±28		372±26		0.63	S
B. longirostris		55±0.5		90±6		1.64	S
H. gibberum		909±77		157±14		0.17	S

vessels, so that the pressure of their predation was removed from such forms as *E. lacustris* and *M. edax*. Filtering organisms such as *Bosmina* tended to increase more in the vessels than in the lake epilimnion. The community was protected against the predation of vertically migrating copepods, e.g. *C. b. thomasi* and *C. vernalis*. Adults of these species were rarely seen in lake samples taken from 0–4 m, but often appeared in vessels following incubation. Apparently, only immature forms of these species occur in the upper epilimnion during daytime collection times. *Bosmina* and other prey organisms would be subject to their predation only after transformation to more mature copepodid forms had occurred.

The biomass of both the entire crustacean community and individual species decreased when exposed to 1 and 3 $\mu g \cdot L^{-1}$ Cd and pH 6.7–6.8. The response to cadmium of the total community and of individual species generally decreased as the pH of the lake was lowered (Table 4). Peterson et al. (1984) and Peterson and Healey (1985) examined the response of algal uptake systems for inorganic nutrients to cadmium at pH 5–8. They found that the amount of cadmium necessary to cause 50% inhibition of uptake was up to 20 000 times higher at pH 5 than at pH 8. This reduced toxicity at low pH is apparently caused by H^+ occupation of cellular binding sites. This occupation decreases the ability of cadmium to compete for such sites (Peterson et al. 1984). A similar relationship exists between free cadmium and cationic calcium. Calcium is a major cation in many lakes and is important physiologically in the molt cycle of crustaceans. In an assessment of cadmium toxicity to the freshwater crustacean, *Gammarus pulex*, Wright (1980) suggested that the calcium regulatory mechanism is the chief mechanism controlling cadmium uptake. As lakes are increasingly acidified H^+ may act in a similar manner to calcium.

Free cadmium ions (Cd^{+2}) are generally considered to be the major toxic species (Petersen et al. 1984; Sunda et al. 1978). Metal-organic complexes are less toxic (Giesy et al. 1977). The planktonic crustacea of lakes are apparently partially protected from the toxic effects of cadmium when elevated concentrations of organic ligands enter the water column. This may occur as the result of biotic metabolism or through the mixing of sediments, hypolimnetic and epilimnetic water in lakes during spring or fall turnover. In 1982, DOC levels in Lake 302S were up to four times higher than in other years and the various major groups (Table 5) exhibited generally lesser responses to cadmium during that year.

The responses of zooplankton communities and various species to additions of cadium *in situ* have been assessed in three kinds of impoundment systems: 10^5 L limnocorrals in ELA Lake 223 (Marshall and Mellinger 1980; Lawrence 1980); small static 8-28 L impoundments incubated in Lake Michigan and in ELA Lake 382 (Marshall and Mellinger 1980; Marshall et al. 1981); and our 41 L flow-through impoundment system in ELA Lake 302S.

The same relative reductions in density of the entire crustacean community were identified in cadmium-enriched impoundments when limnocorrals or the smaller flow-through systems were used as test vehicles. *Bosmina longirostris* and cyclopoid copepods were found to be susceptible to 1 and 3 $\mu g \cdot L^{-1}$ Cd in both systems, but calanoid copepods were affected only in the flow-through system. Crustacean density in limnocorrals enriched with 1–30 $\mu g \cdot L^{-1}$ Cd did not vary in 24 d after addition (Marshall and Mellinger 1980). The half-life of cadmium in these tubes was 11–50 d (D.W. Schindler, unpublished data), so that the change in response to cadmium took place at concentrations decreasing at different rates.

Reduction of the densities of the total crustacean community and of *B. longirostris*, *T. p. mexicanus*, and cyclopoid copepodids C1-5 occurred in the small sealed impoundments enriched with cadmium at concentrations of 0.6–5.0 $\mu g \cdot L^{-1}$ Cd (Marshall and Mellinger 1980; Marshall et al. 1981). The reductions were marked only with additions of 5 $\mu g \cdot L^{-1}$ Cd except for that of *H. gibberum* which was eliminated at all concentrations. These impoundments were not designed to exchange with the lake environment. It is likely that build up of metabolites and particulate debris in the impounded community during the experimental period contributed to the formation of organic materials which complex with cadmium and which decrease its toxic action (Giesy et al. 1977). By extrapolation of their results, Marshall and Mellinger (1980) suggested that density of crustaceans will respond to concentrations as low as 0.2 $\mu g \cdot L^{-1}$ Cd. Our biomass results confirm this suggestion. Further, we predict that the biomass of the most sensitive zooplankton in these lakes, *H. gibberum*, will be affected by concentrations at or below 0.2 $\mu g \cdot L^{-1}$ Cd, the present maximum allowable concentration for the protection of aquatic life in the soft water lakes of Canada (Reeder et al. 1979).

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Limnocorral Studies of Chemical and Biological Acid Neutralization in Two Freshwater Lakes

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Schiff, S. L., and R. F. Anderson. 1987. Limnocorral studies of chemical and biological acid neutralization in two freshwater lakes. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 173–187.

Twenty-nine 1 m diameter limnocorrals were installed in two Canadian Shield lakes to examine the relative importance of individual acid neutralization processes. Once the water column alkalinity was depleted, the within-lake alkalinity production processes were confined to the lake sediments. The flocculent organic-rich sediments of a shallow dystrophic lake (Lake 114) neutralized acid at a significantly greater rate than the sandy littoral sediments of an oligotrophic lake (Lake 302). Since the water column alkalinity of Lake 114 is much lower than Lake 302, ambient alkalinity is not a good predictor of acid neutralization capacity in the sediments. In both lakes, HNO_3 was neutralized at a substantially higher rate than HCl or H_2SO_4. The rates of nitrate consumption, sulfate consumption, and abiotic acid neutralizing processes are independent of each other. Denitrification to an uncharged end-product is the primary mechanism of NO_3^- removal. The principal source of non-anion-specific buffering in the enclosure experiments was Ca^{2+} release from the sediments in Lake 302 and Ca^{2+} and NH_4^+ release in Lake 114. There is no significant difference between the organic-rich sediments of Lake 114 and the sandy sediments of Lake 302 in the concentration of exchangeable cations ($\mu eq \cdot cm^{-3}$) for all cations except NH_4^+ and Mn^{2+}. Alkalinity production in epilimnetic sediments is an important component of the resistance of softwater lakes to anthropogenic acidification.

Vingt-neuf enceintes limnologiques de 1 m de diamètre ont été plaçées dans deux lacs du bouclier canadien afin d'étudier l'importance relative de divers processus de neutralisation de l'acidité. Après disparition de l'alcalinité de la colonne d'eau, les processus de génération d'alcalinité se trouvaient confinés aux sédiments lacustres. Les sédiments de floculation riches en matières organiques d'un lac dystrophe peu profond (lac 114) neutralisaient l'acidité à un taux significativement plus élevé que les sédiments de la rive sableuse d'un lac oligotrophe (lac 302). L'alcalinité de la colonne d'eau du lac 114 étant de beaucoup inférieure à celle du lac 302, l'alcalinité ambiante ne constitue pas un bon indice de prévision de la capacité de neutralisation de l'acidité dans les sédiments. Dans les deux lacs, le HNO_3 était neutralisé à une vitesse passablement plus élevée que le HCl ou le H_2SO_4. Les taux d'utilisation du nitrate et du sulfate de même que les processus abiotiques de neutralisation de l'acidité sont tous indépendants les uns des autres. La dénitrification d'un produit final non ionisé constitue le premier mécanisme d'élimination du NO_3^-. Au cours des essais en enceintes, l'effet tampon non spécifiquement anionique avait pour cause principale le Ca^{2+} libéré des sédiments, dans le lac 302, et le Ca^{2+} et le NH_4^+ libérés, dans le lac 114. À l'exception des cations NH_4^+ et Mn^{2+}, la concentration des cations échangeables ($\mu eq \cdot cm^{-3}$) ne différait pas de façon significative entre les sédiments riches en matières organiques du lac 114 et les sédiments sableux du lac 302. La génération d'alcalinité par les sédiments épilimnétiques joue un rôle important dans la résistance des lacs à eau douce à l'acidification anthropogène.

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Acidic deposition in northeast North America is composed of approximately 70% sulfuric acid and 30% nitric acid. Emission regulation strategies to protect freshwater lakes have focussed on reduction of SO_x emissions rather than NO_x emissions because of evidence for acidification of freshwater lakes by H_2SO_4 (eg. Schindler et al. 1980, 1985a). Both sulfuric and nitric acids may be neutralized by natural biogeochemical processes in lakes. Significant sulfate consumption, which results in neutralization of H_2SO_4 (reaction 5: Table 1), occurs only in anaerobic sediments. In contrast, nitrate is a nutrient for autotrophic plankton and periphyton. Both assimilation of nitrate in the water column during photosynthesis (the reverse of reaction 2, Table 1) and denitrification of NO_3^- in the sediments (reactions 3a, b, c; Table 1) results in neutralization of HNO_3. Denitrification is also energetically favoured in comparison with SO_4^{2-} reduction. Consequently, unlike H_2SO_4, evidence for acidification of freshwater lakes by HNO_3 is sparse and generally confined to discrete events such as snowmelt.

In addition to nitrate and sulfate consumption, several other important acid neutralizing processes occur in lake sediments including microbial reduction of Mn(IV) and Fe(III) oxyhydroxides (reactions 4 and 5), cation exchange (reaction 7), organic acid protonation (reaction 8), and sediment weathering (reaction 9).

In lakes impacted by acidic precipitation, whole-lake input–output budgets are unable to directly quantify the importance of each of these sedimentary processes (listed in Table 1) primarily due to uncertainties in dry deposition, water budget and fluxes of gaseous phase species (Wright 1983;

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TABLE 1. Stoichiometry of Redfield (Redfield et al. 1963) organic matter oxidation with various terminal electron acceptors (written for neutral pH). See also Froelich et al. (1979); Emerson et al. (1980); Kuivila and Murray (1984).

1) Aerobic respiration — including nitrification:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 138\ O_2 \rightarrow 106\ CO_2 + 16\ NO_3^- + HPO_4^{-2} + 122\ H_2O + 18\ H^+$	$\Delta Alk/O_2$ (eq/mole) = −18/138[a]
2) Denitrification:	
a) With oxidation of organic nitrogen[b]:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 94.4\ NO_3^- + 92.4\ H^+ \rightarrow 106\ CO_2 + 55.2\ N_2 + HPO_4^{-2} + 177.2\ H_2O$	$\Delta Alk/\Delta NO_3^-$ (eq/eq) = +92.4/94.4
b) Without oxidation of organic nitrogen:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 84.8\ NO_3^- + 98.8\ H^+ \rightarrow 106\ CO_2 + 42.4\ N_2 + 16\ NH_4^+ + HPO_4^{-2} + 148.4\ H_2O$	$\Delta Alk/\Delta NO_3^-$ (eq/eq) = +98.8/84.8
c) Ammonia production	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 53\ NO_3^- + 120\ H^+ \rightarrow 106\ CO_2 + 69\ NH_4^+ + HPO_4^{2-} + 53\ H_2O$	$\Delta Alk/\Delta NO_3^-$ (eq/eq) = +120/53
3) MnO_2 Reduction:	
a) With oxidation of organic nitrogen:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 236\ MnO_2 + 470\ H^+ \rightarrow 236\ Mn^{+2} + 106\ CO_2 + 8\ N_2 + HPO_4^{-2} + 366\ H_2O$	$\Delta Alk/\Delta Mn^{2+}$ (eq/eq) = +470/472
b) Without organic nitrogen oxidation:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 212\ MnO_2 + 438\ H^+ \rightarrow 212\ Mn^{+2} + 106\ CO_2 + 16\ NH_4^+ + HPO_4^{-2} + 318\ H_2O$	$\Delta Alk/\Delta Mn^{2+}$ (eq/eq) = +438/424
4) Fe (III) Reduction:	
$(CH_2O)_{106}(NH_3)_{16}\ (H_3PO_4) + 424\ FeOOH + 862\ H^+ \rightarrow 424\ Fe^{+2} + 106\ CO_2 + 16\ NH_4^+ + HPO_4^{-2} + 742\ H_2O$	$\Delta Alk/\Delta Fe^{2+}$ (eq/eq) = +862/848
5) SO_4^{2-} Reduction:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 53\ SO_4^{-2} + 67\ H^+ \rightarrow 106\ CO_2 + 16\ NH_4^+ + 53\ HS^- + HPO_4^{-2} + 106\ H_2O$	$\Delta Alk/\Delta SO_4^{2-}$ (eq/eq) = +120/106
6) CH_4 Fermentation:	
$(CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 14\ H^+ \rightarrow 53\ CO_2 + 53\ CH_4 + 16\ NH_4^+ + HPO_4^{-2}$	$\Delta Alk/\Delta CH_4$ (eq/mole) = +14/53
7) Cation Exchange:	
$X{-}M^{+n} + nH^+ \rightarrow X{-}n(H^+) + M^{+n}$	
X = Mineral or organic substrate; $M = Ca^{+2}, Mg^{+2}, Na^+, K^+, NH_4^+$, etc.	$\Delta Alk/\Delta M^{n+}$ (eq/eq) = +1/1
8) Organic Acid Protonation:	
$R{-}COO^- +\ H + \rightarrow R{-}COOH$	
9) Mineral Weathering:	
e.g. $CaAl_2Si_2O_8 + H_2O + 2\,H^+ \rightarrow Ca^{+2} + Al_2Si_2O_5(OH)_4$	$\Delta Alk/\Delta Ca^{2+}$ (eq/eq) = +1/1

[a] ΔAlk convention: negative ≡ Alk consumed; positive ≡ Alk generated.
[b] Note: Alk is consumed due to nitrification.

Jeffries et al. 1984). In addition, the within-lake production and consumption terms are small compared to inputs and outputs for many components (Cook et al. 1986; Schindler et al. 1986), making it difficult to calculate within-lake acid neutralization from whole-lake mass budgets of major ions.

In this work, limnocorral experiments were designed to separate and quantify the relative importance of biotic and abiotic acid neutralization processes in two softwater lakes, and to test the applicability of the reaction stoichiometries listed in Table 1 to the assessment of the effects of acidic precipitation.

Experimental Design and Theory

To isolate the effects of nitric and sulfuric acids, several small enclosures were constructed in two small Precambrian shield lakes. Two lakes of contrasting sediment characteristics were chosen, enabling a comparison of acid neutralization in sediments of high and low organic matter content (Table 2).

Sets of four enclosures (tubes) were manipulated as follows. One tube in each set received additions of either HCl, HNO_3 or H_2SO_4. The fourth tube served as a control and received no chemical additions. The pH of each experimental tube was adjusted to a target pH between 5.0 and 5.2. This pH range has been suggested as a lower limit for successful reproduction in many forms of aquatic wildlife (e.g. Overrein et al. 1981; Schindler et al. 1985a) and is comparable to ongoing whole lake acidification projects at ELA. Periodic acid additions to the enclosures were required to maintain a pH of 5.0–5.2,

TABLE 2. Selected lake and lake sediment characteristics.

Characteristics	Lake 114	Lake 302
Area ($10^4 m^2$)	12.1	10.9
Z_{max} (m)	5.0	10.6
Mean Z (m)	1.7	5.1
Water column[a]:		
alkalinity ($\mu eq \cdot L^{-1}$)	27.0	72.0
pH	5.9	6.7
SO_4^{2-} ($\mu eq \cdot L^{-1}$)	92.0	58.0
DOC ($mgC \cdot L^{-1}$)	9.6	6.0
conductivity ($\mu S \cdot cm^{-1}$)	22.0	30.0
Sediment:		
organic content[b]	24%	1.6%
surficial porosity	0.99	0.70
cation exchange capacity[c] (meq/100 g)	8.4	0.42

[a] Epilimnetic values at time of enclosure deployment.
[b] Kelly and Rudd (1984).
[c] Calculated from the sum of the cations released with 0.1 N LiCl (Table 4).

indicating that H^+ was being consumed. Total acid consumption by all processes therefore equals the acid added in each experimental enclosure.

Because Cl^- concentrations are not mediated by any known biological cycles, the HCl required to maintain the target pH in the HCl tube is the sum of the water column alkalinity and the non-anion-specific sediment buffering such as cation exchange, sediment weathering, or protonation of organic acids. Physical transport of H^+ into the sediments by diffusion or bioturbation is included in the HCl consumption term. Acid required to maintain the pH of 5.0–5.2 in the H_2SO_4 or HNO_3 enclosures in excess of the acid added to the HCl enclosure should then correspond to the additional acid neutralization associated with biological consumption of SO_4^{2-} or NO_3^-. One additional open-bottom tube in each lake was acidified with HCl, and the SO_4^{2-} and NO_3^- anions were added as Na salts, to examine whether the two biological cycles act independently.

To isolate buffering processes in the water column from those in the sediments, a set of four tubes with closed-bottoms (water only) in Lake 302 was manipulated in a manner analagous to the open-bottom tubes (water and sediments) in Lake 302.

Analysis of water column chemical data was used to identify alkalinity producing processes occurring in the water column and sediments. Alkalinity is the excess of weak acidic anions over protons. In oxic water columns of typical Canadian Shield lakes:

$$(1) \qquad ALK = [HCO_3^-] + [\text{organic acid anions}] - [H^+]$$

where the concentration units in square brackets are expressed in equivalents per litre. If the organic acid anionic contribution to alkalinity remains constant over the course of the experiment, and since at pH 5.2 $[HCO_3^-]$ is negligible, then the acid added (AA) equals the initial water column alkalinity (ALK) plus the alkalinity production (ΔALK_1). Therefore, the total alkalinity production can be calculated:

$$(2) \qquad \Delta ALK_1 = AA - ALK$$

Alternatively, alkalinity can be defined as the excess of the sum of strong basic cations over strong acidic anions:

$$(3) \qquad ALK = [Ca^{2+}] + [Mg^{2+}] + [K^+] + [Na^+] + [NH_4^+] + [Mn^{2+}] - [SO_4^{2-}] - [Cl^-] - [NO_3^-]$$

Any observed increase in the sum of strong basic cations less the sum of strong acidic anions provides an independent measure of alkalinity production in the enclosures:

$$(4) \qquad \begin{aligned} \Delta ALK_2 = {} & \Delta[Ca^{2+}] + \Delta[Mg^{2+}] + \Delta[K^+] + \Delta[Na^+] \\ & + \Delta[NH_4^+] + \Delta[Mn^{2+}] - \Delta[SO_4^{2-}] \\ & - \Delta[Cl^-] - \Delta[NO_3^-] \\ = {} & \Delta\text{cations} - \Delta\text{anions} \end{aligned}$$

In the enclosure experiments, the total acid neutralization is calculated by two independent methods: (1) the total acid neutralization equals the total amount of acid added to each enclosure to maintain the target pH of 5.0–5.2 (AA), and (2) the total acid neutralization equals AN, the initial water column alkalinity (ALK) plus the change in time of each of the ions ($\Delta ALK_2 = \Delta\text{cations} - \Delta\text{anions}$):

$$(5) \qquad AN = ALK + \Delta ALK_2$$

If:

$$(6) \qquad AA = AN$$

then all the individual contributors to acid neutralization have been correctly identified and quantified. Only the overall stoichiometry of the net reaction sequence can be determined from the water column data and the processes responsible for acid neutralization must be inferred.

Two other experimental enclosures were installed in each lake to examine the effects of NH_4Cl and $CaCl_2$ additions. A portion of the sulfate in wet deposition is neutralized by NH_3 to form the salt $(NH_4)_2SO_4$. Ammonium ion is an important component of bulk deposition, in some months approximately equalling the NO_3^- deposition in acid-stressed areas near Dorset, Ontario (Scheider et al. 1979). Phytoplankton uptake of NH_3 or nitrification of NH_4^+ to N_2 or N_2O regenerates the strong acid H_2SO_4 from $(NH_4)_2SO_4$. To examine the acidifying effect of NH_4^+, NH_4Cl was added to one open-bottom enclosure in each lake. Chloride was chosen as the NH_4^+ counter-ion to avoid complexity arising from simultaneous alkalinity production as a result of the consumption of the SO_4^{2-} counter-ion.

One mitigation strategy for acid-stressed lakes is whole-lake treatment with lime. Calcium chloride was added to one open-bottom tube in each lake to examine the effects of increased dissolved calcium concentrations, especially with respect to ion-exchange of protons, major cations, and trace metals.

Materials and Methods

Twenty-nine limnocorrals (tubes) were deployed in two lakes between 1981 and 1983 (Table 3). Lake 302 is an oligotrophic lake with littoral sediments of coarse sand overlain by a thin (<1 cm) flocculent organic layer. Lake 114 is a shallow dystrophic lake with flocculent organic-rich sediments. The sediments of both lakes are anoxic within 1–2 cm of the sediment-water interface (Kelly and Rudd 1984). Neither of these two lakes have $CaCO_3$ present in the sediments. Sediments of nearby Lake 239 are composed of plagioclase, quartz, and K-feldspar with secondary minerals: kaolinite, chlorite, illite, and Ca-montmorillonite (Brunskill et al. 1971). Lake 302 and Lake 114 are part of the whole lake acidification projects ongoing at the Experimental Lakes Area (ELA) in NW Ontario (Schindler 1985). Sulfuric acid has been added to Lake 114 since 1979 at a loading rate equivalent to an annual precipitation of pH 4.0. The limnocorrals in Lake 302 were installed prior to any artificial addition of acid to the lake. Salient lake and lake sediment characteristics are listed in Table 2.

The limnocorrals were constructed of nylon reinforced polyethylene and measured 1 m in diameter and 1.2 m in water depth. A wooden frame equipped with styrofoam floats supported the tubes at approximately 30 cm above the water surface. The bottoms of the tubes were either embedded in the sediment (open-bottom tubes), permitting chemical exchange between the water column and sediments, or sealed with an attached watertight polyethylene bottom (closed-bottom tubes) to isolate the water column and prevent sediment–water exchange. The exposed sediment area in each open-bottom tube is a constant since it is controlled by a rigid 1 m diameter support which penetrates and encloses the sediments.

Tritium was added to each tube in 1982 and 1983 to determine the initial volume and to assess leakage. The error in the

TABLE 3. Enclosure treatments.

Year	Tube	Lake	Treatment	Target pH	Open/Closed bottom	Notes[b]
1981	A1	114	Control	NC[a]	O	1
	A2	114	HCl	5.0–5.2	O	1
	A3	114	HNO_3	5.0–5.2	O	1
	A4	114	H_2SO_4	5.0–5.2	O	1
1982	B1	302	Control	NC	O	
	B2	302	HNO_3	5.0–5.2	O	
	B3	302	HCl	5.0–5.2	O	
	B4	302	H_2SO_4	5.0–5.2	O	
	B5	302	$HCl + NaNO_3 + Na_2SO_4$	5.0–5.2	O	2
	B6	302	NH_4Cl	NC	O	3
	B7	302	$CaCl_2$	NC	O	4
	B8	302	Control	NC	C	
	B9	302	HNO_3	5.0–5.2	C	
	B10	302	HCl	5.0–5.2	C	
	B11	302	H_2SO_4	5.0–5.2	C	
	B12	114	Control	NC	O	5
	B13	114	HNO_3	5.0–5.2	O	
	B14	114	HCl	5.0–5.2	O	
	B15	114	H_2SO_4	5.0–5.2	O	6
	B16	114	$HCl + NaNO_3 + Na_2SO_4$	5.0–5.2	O	6
	B17	114	NH_4Cl	NC	O	7
	B18	114	$CaCl_2$	NC	O	4, 5
	B19	114	Control	NC	O	
	B20	114	$HCl + NaNO_3 + Na_2SO_4 + H_3PO_4$	5.0–5.2	O	8
	B21	114	$HCl + NaNO_3 + Na_2SO_4 + H_3PO_4$	5.0–5.2	O	9
1983	C1	114	Control	NC	O	
	C2	114	HNO_3	5.0	O	
	C3	114	HCl	5.0	O	
	C4	114	H_2SO_4	5.0	O	

[a] NC = pH not manipulated.

[b] Notes: 1, Also maintained target pH with acid additions in 1982. 2, $NaNO_3 + Na_2SO_4$ added at same loading rate as HNO_3 in B2 and H_2SO_4 in B4, respectively. 3, NH_4Cl added at same loading rate in moles of N as HNO_3 in B2 until 26 July. 4, 20 g $CaCl_2$ added at beginning of experiment. 5, 20 g $CaCl_2$ added 14 Sept. 1982. 6, $NaNO_3 + Na_2SO_4$ added at same rate as HNO_3 in B13 and Na_2SO_4 in B15, respectively. 7, NH_4Cl added at same loading rate in moles of N as HNO_3 in B13 until 26 July. 8, Same as B16 with 0.011 g of P added as H_3PO_4 per week. 9, Same as B16 with 0.022 g of P added as H_3PO_4 per week.

initial volume is less than 5% for 1982 and less than 2% for the 1983 experiments. The volume estimates are used to convert the loading of acid or salts to $\mu eq \cdot L^{-1}$. These initial volumes cannot be subsequently corrected for evaporation or dilution because of the uncertainty in the rate of ^3H escape to the atmosphere over the course of the experiment. Leakage of one individual tube can, however, be assessed by comparing the ^3H loss rate with other tubes.

The pH of the tubes was monitored in the field using a portable Radiometer pH meter with a combination electrode and calibrated in the field with Fisher standard buffers at pH 7.0 and pH 4.0. The water column pH was then adjusted to a target pH between 5.0 and 5.2 with 0.1 N solution of the appropriate reagent–grade acid. The tubes were monitored every 2–3 d during the summer stratification period. Acid was never added more frequently than on a daily basis or in sufficient quantity to lower the pH significantly below pH 5.0. This prevented the pH dipping below the target pH and subsequent "acid shock" to pH-sensitive organisms. Convective mixing occurred when surface waters cooled each night. The tubes were never mixed mechanically to avoid resuspension of flocculent surficial sediments.

Weekly water samples integrated over the top 1 m water column were collected early in the morning and analyzed for major cations and anions, trace metals, ^3H, NH_4^+, and NO_3^-. Major cations were quantified by flame atomic absorption spectroscopy and plasma emission spectroscopy. Trace metals were analyzed by graphite furnace AA. Blank corrections were significant only for Zn (10–30%). Precision for the cation and trace metal analyses were as follows; Ca^{2+}: 2%, Mg^{2+}: 3%, Na^+: 1.5%, K^+: 4%, Mn: 5%, Fe: 10–15%, Al: 20%, and Zn: 10–15%. SO_4^{2-} and Cl^- were measured on a Dionex ion chromatograph with an average precision of 1.5 and 5%, respectively. NH_4^+, NO_3^-, TDN, and chlorophyll *a* were analyzed using standard techniques of Stainton et al. (1977). In 1983, alkalinites were measured by the Gran titration technique with a precision of ±2%.

Sediment porewater profiles were collected with an in situ porewater sampler (Hesslein 1976). The syringes used for sample collection were pre-filled with N_2 to prevent the introduction of O_2 into the samples.

Exchangeable cations in the lake sediments were determined with 1.0 N solutions of LiCl and $CaCl_2$ in a nitrogen glove box, following the method of Boatman and Murray (1982).

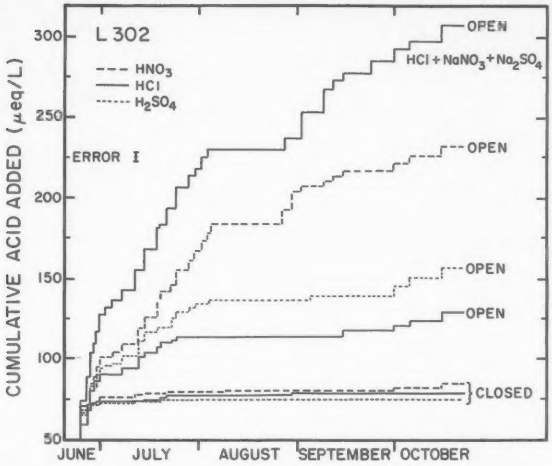

FIG. 1. Cumulative acid additions necessary to maintain pH 5.0–5.2 in open and closed-bottom tubes in Lake 302. There was a 2-wk period in August during which the tubes were not monitored for logistical reasons.

Results and Discussion

In the following section, a series of different enclosures will be compared to determine whether the acid is neutralized in the water column or sediments, to quantify biotic versus abiotic acid neutralization, to determine whether or not neutral salts can have an acidifying effect, and to contrast the effect of sediment type on the results.

1. Importance of Sedimentary Processes: Lake 302

In each of the closed-bottom tubes, approximately 77 $\mu eq \cdot L^{-1}$ of acid were necessary to neutralize the enclosed water column alkalinity (Fig. 1). Further additions were seldom required to maintain the pH of 5.0–5.2 (Fig. 1 and 2). Thus, once the water column alkalinity is consumed, acid neutralization by processes in the water column is negligible.

To maintain the target pH, the Lake 302 open-bottom tubes consumed acid in the order: $HNO_3 > H_2SO_4 > HCl$ (Fig. 1). The addition of the salts $NaNO_3$ and Na_2SO_4 to a tube which was acidified with HCl required the greatest amount of acid (Fig. 1). Since all acids behaved similarly in the closed-bottom tubes, the differences in acid consumption observed in the open-bottom tubes involve anion-specific, sediment-coupled mechanisms.

Comparison of the closed and open-bottom tubes illustrates the acid buffering by sedimentary processes. After 2 months, the open-bottom HCl tube required approximately 1.5 times as much acid to maintain the same pH as the closed-bottom HCl tube (Fig. 1). This additional acid was consumed by abiotic acid neutralization processes in the sediments such as cation exchange, sediment weathering or protonation of organic acid anions and the diffusion of the acid into the sediments. In the H_2SO_4 open-bottom tube, the amount of acid added after 2 months was approximately 1.8 times the amount added to the closed-bottom tube, demonstrating the additional effect of sedimentary processes consuming the SO_4^{2-} counter-ion. The open-bottom HNO_3 tube neutralized 2.4 times the acid as the corresponding closed-bottom tube in the same 2-month period, emphasizing the effect of sedimentary buffering processes consuming nitrate.

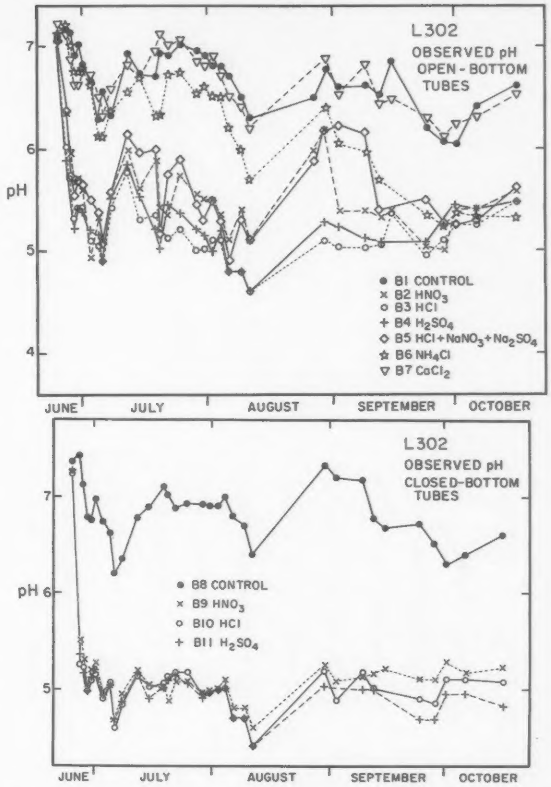

FIG. 2. Temporal pattern of observed water column pH in open and closed bottom tubes in Lake 302. The pH measurements are obtained immediately prior to the addition of acid and are therefore elevated above the average water column pH.

2. Abiotic Acid Neutralizing Processes: Lake 302

Comparison of cation/anion budgets with the acid additions aids in delineating the acid buffering mechanisms. Acid neutralization in the open-bottom HCl tube (AN_{Cl}) is derived from two sources: neutralization of the water column alkalinity (ALK) and exchange for cations in the sediments ($\Sigma\Delta$Cations).

$$AN_{Cl} = ALK + \Sigma\Delta\text{Cations} \tag{7}$$

ALK is equivalent to the acid added to the closed-bottom HCl tube. $\Sigma\Delta$Cations is calculated from the sum of the concentration increase of each major cation in the open-bottom HCl tube compared to the open-bottom control tube, assuming that the alkalinity of the control tube is only affected by evaporation/dilution. The sum of the independently measured contributions to acid neutralization approximately equals the amount of HCl added within the uncertainty (Fig. 3), indicating that the major acid neutralization processes in the HCl enclosure have been correctly identified and quantified.

TABLE 4. Exchangeable cations in lake sediments.

	Lake 302		Lake 114		Lake 302		Lake 114	
	LiCl (μeq/g)[a]	$CaCl_2$ (μeq/g)	LiCl (μeq/g)	$CaCl_2$ (μeq/g)	LiCl (μeq/cm^3)[b]	$CaCl_2$ (μeq/cm^3)	LiCl (μeq/cm^3)	$CaCl_2$ (μeq/cm^3)
Na^+	0.05 ± .01	0.016 ± .003	0.5 ± .2	0.6 ± .1	0.038 ± .007	0.012 ± .002	0.013 ± .004	0.014 ± .003
K^+	.011 ± .01	0.06 ± .03	1.9 ± .5	1.28 ± .08	0.10 ± .01	0.05 ± .02	0.05 ± .01	0.032 ± .002
Mg^{2+}	0.41 ± .06	—	10.6 ± 0.9	10.3 ± 0.5	0.31 ± .05	—	0.27 ± .02	0.26 ± .01
Ca^{2+}	1.8 ± .3	—	53. ± 4	—	1.4 ± .2	—	1.4 ± .1	—
Fe^{2+}	0.44 ± .12	1.1 ± .5	13.4 ± 1.3	16.9 ± 1.6	0.33 ± .09	0.75 ± .38	0.33 ± .03	0.44 ± .04
Mn^{2+}	1.4 ± .2	2.25 ± .05	0.89 ± .08	0.95 ± .03	1.1 ± .1	1.70 ± .04	0.022 ± .02	0.024 ± .001
NH_4^+	0.060 ± .02	0.07 ± .01	3.6 ± .5	3.7 ± .3	0.05 ± .01	.05 ± .01	0.09 ± .01	0.092 ± .008

[a] μeq/g (dry weight of sediments).
[b] μeq/cm^3 (wet volume of sediments).

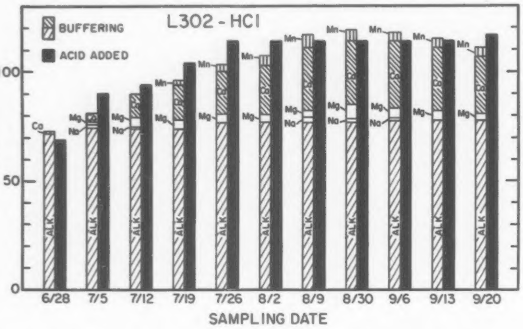

FIG. 3. Comparison of total acid added and the individual contributions to acid neutralization in the HCl open-bottom tube in Lake 302. Buffering by cation exchange is calculated as the difference between the concentration of each cation in the HCl tube and in the control tube.

The major cation exchanged for the added H^+ is Ca^{2+}; Mg^{2+} and Mn are minor contributors, and exchanges of Na^+, K^+, NH_4^+, Fe, and Al are negligible. The concentration of exchangeable cations determined in a laboratory cation-exchange experiment follows the order observed for water column accumulation: $Ca^{2+} > Mg^{2+} >> K^+ > Na^+$ (Table 4). In Lake 302, there is a large supply of ion-exchangeable Fe^{2+} and Mn^{2+}. However, Fe^{2+} diffusing from the bulk sediment will be re-oxidized at the anoxic–oxic boundary and cannot accumulate in the oxic water column. Mn oxidation at pH 5 is extremely slow and Mn^{2+} was observed in the Lake 302 tubes in approximately the same concentration as Mg^{2+} (Fig. 3).

3. Acid Neutralization by SO_4^{2-} Consumption: Lake 302

To maintain the target pH, a larger quantity of acid was added to the H_2SO_4 tube than to the HCl tube (Fig. 1). The increase in water column SO_4^{2-} was less than the H_2SO_4 added (Fig. 4), indicating that SO_4^{2-} was consumed. Total acid neutralization in the H_2SO_4 tube is:

$$(8) \qquad AN_{SO_4} = ALK + \Sigma\Delta Cations - \Delta SO_4^{2-}$$

where ALK and ΣΔCations are as above, and ΔSO_4^{2-}, the amount of added SO_4^{2-} which is consumed, is the difference between the measured SO_4^{2-} increase in the water column and the H_2SO_4 added. The individual contributions to the ΣΔCations term for the H_2SO_4 tube were measured and do not differ significantly from the HCl tube (data not shown), indicating that abiotic and biotic processes are independent of the acidic counter-ion. Since the amount of acid added to all the closed-bottom tubes is identical, ALK (water column alkalinity) is also equal in the HCl and H_2SO_4 tubes. Thus, equations (7) and (8) can be combined:

$$(9) \qquad AN_{SO_4} = AN_{Cl} - \Delta SO_4^{2-}$$

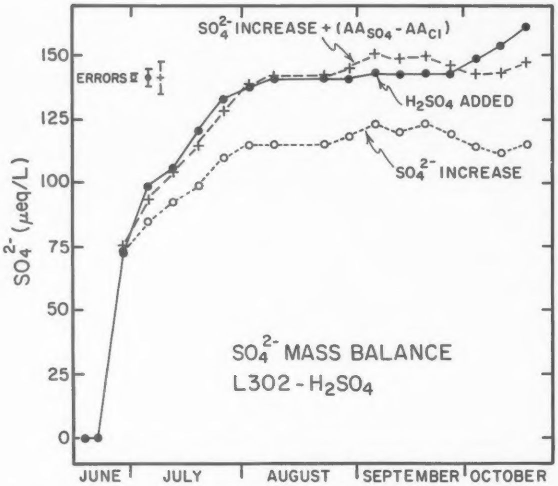

FIG. 4. Sulfate mass balance in H_2SO_4 tube in Lake 302. "SO_4^{2-} increase" is the SO_4^{2-} concentration measured on each sampling date minus the initial SO_4^{2-} concentration. "H_2SO_4 added" is the cumulative sum of the H_2SO_4 additions to the tube. SO_4^{2-} + (AA_{SO_4} − AA_{Cl}) is the "SO_4^{2-} increase" plus the difference between the cumulative H^+ added to the H_2SO_4 tube and the H^+ added to the HCl tube. Average errors include uncertainty in enclosure volume and analytical errors.

When the difference between the H_2SO_4 and HCl cumulative acid additions, (AA_{SO_4} − AA_{Cl}), is added to the measured increase in SO_4^{2-}, the sum matches the added H_2SO_4 within the experimental error (Fig. 4). Thus, the disappearance of one equivalent of SO_4^{2-} from the water column is balanced by

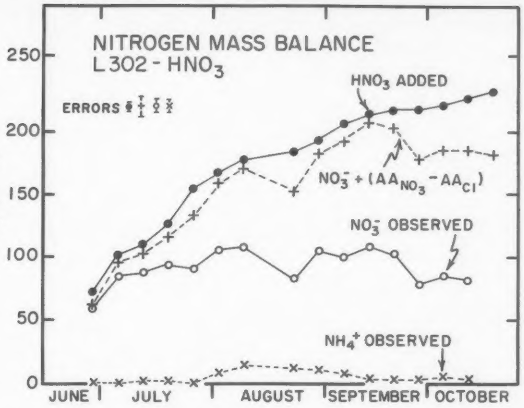

FIG. 5. Nitrate mass balance in HNO_3 tube in Lake 302. "HNO_3 added" is the cumulative sum of HNO_3 additions to the tube. NO_3^- + ($AA_{SO_4} - AA_{Cl}$) is the observed water column NO_3^- plus the difference between the H^+ added to the HNO_3 and the H^+ added to the HCl tube. Initial water column NO_3^- concentration was negligible.

consumption of one equivalent of H^+. The good agreement between the total acid neutralized in the H_2SO_4 tube and the sum of the three independently calculated contributions to alkalinity production (ALK, $\Sigma\Delta$Cations, SO_4^{2-}) indicates that all the acid neutralization is accounted for by these three processes.

The fate of the consumed SO_4^{2-} cannot be unequivocally identified in these experiments since adsorption of SO_4^{2-} to Fe oxides (which are present in the surficial Lake 302 sediments), loss of H_2S to the atmosphere, or storage of sulfur as S^0, iron sulfide, pyrite, or organic sulfur will each yield the same net 1:1 ratio (in equivalents) of $H^+:SO_4^{2-}$ consumption in the water column of the tube experiments (Anderson and Schiff 1987).

4. Acid Neutralization by NO_3^- Consumption: Lake 302

Of the three acids added to tubes in Lake 302, nitric acid had the lowest efficiency of acidification (Fig. 1). In a treatment analogous to the H_2SO_4 tube, the sum of the observed acid neutralization processes in the HNO_3 tube is:

$$(10) \qquad AN_{NO_3} = ALK + \Sigma\Delta Cations - \Delta NO_3^-$$

The NO_3^- observed is substantially less than the HNO_3 added (Fig. 5). The ALK and $\Sigma\Delta$Cations terms are the same for the HNO_3 and HCl tubes (data not shown) and:

$$(11) \qquad AN_{NO_3} = AN_{Cl} - \Delta NO_3^-$$

When (AA_{NO_3} = AA_{Cl}) is added to the observed NO_3^-, the sum matches the HNO_3 added (Fig. 5). This agreement indicates that the excess acid demand in the HNO_3 tube was due to NO_3^- consumed in a ratio with H^+ (H^+: NO_3^-) of 1:1. Either assimilatory uptake (Table 1, reverse of reaction 3a) or denitrification (reaction 3b) satisfies this 1:1 pathway. Dissolved NH_4^+ concentrations in the Lake 302 tubes are negligible, and the effect of a small increase in NH_4^+ in the HNO_3 tube is within the uncertainty of the acid budget calculation.

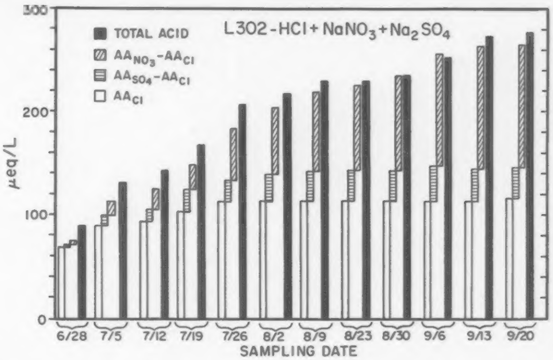

FIG. 6. Cumulative acid added to tube B5 (HCl + $NaNO_3$ + Na_2SO_4) in Lake 302 compared with the individual components of acid consumption evaluated in the HCl, HNO_3, and H_2SO_4 tubes. AA_{Cl} corresponds to the acid neutralization by the water column alkalinity and by non-anion-specific processes in the sediments in the HCl tube. Acid neutralization due to the consumption of SO_4^{2-} in the H_2SO_4 tube is calculated as [$AA_{SO_4} - AA_{Cl}$] and acid neutralization due to consumption of NO_3^- in the HNO_3 tube is calculated as [AA_{NO_3} − AA_{Cl}].

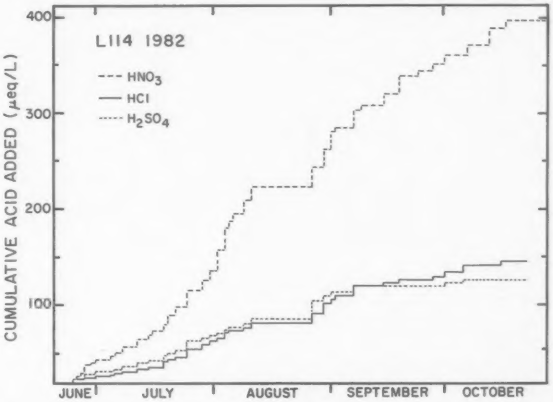

FIG. 7. Cumulative acid additions to maintain a pH of 5.0–5.2 in the HCl, H_2SO_4, and HNO_3 tubes in Lake 114 in 1982. There is a 2-wk period in August when the tubes could not be monitored for logistical reasons.

5. Independence of ΔNO_3^-, ΔSO_4^{2-}, and Abiotic Processes: Lake 302

To ascertain if the acid neutralization processes observed in the individual HCl, H_2SO_4 and HNO_3 tubes are additive, Na salts of NO_3^- and SO_4^{2-} were added to one open-bottom tube in Lake 302 (subsequently referred to as tube B5) at the same loading rate as HNO_3 and H_2SO_4 to the individual acid tubes. The target pH was maintained with HCl. If the effects are additive then:

$$(12) \qquad AN_{B5} = ALK + \Sigma\Delta Cations - \Delta SO_4^{2-} - \Delta NO_3^-$$

More acid was added to the B5 tube than to the HNO_3 or H_2SO_4 tubes individually (Fig. 1). The loading rates of the Na

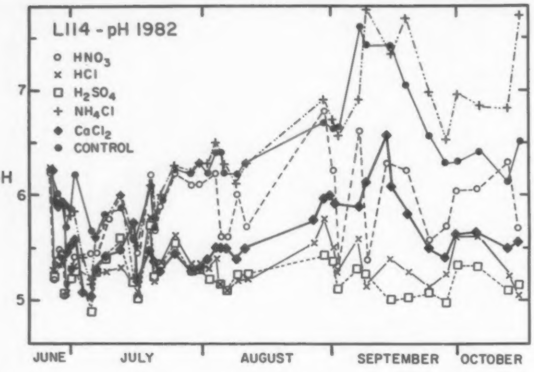

FIG. 8. Temporal pattern of observed water column pH in Lake 114 1982 tubes. The water column pH in the tubes are the maximum in situ values since the pH is recorded immediately prior to acid addition.

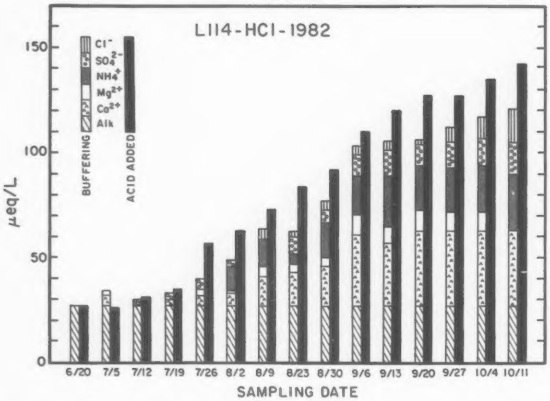

FIG. 9. Comparison of total acid added and the individual contributions to acid neutralization in the Lake 114 HCl tube in 1982. Cation contributions to alkalinity production (including NH_4^+) are calculated as the difference in observed water column concentrations between the HCl tube and the control tube on each sampling day. The SO_4^{2-} contribution is the difference between SO_4^{2-} at beginning of experiment and date of balance because SO_4^{2-} was not measured in the control tube. Acid consumption denoted as "Alk" is the initial water column alkalinity plus the acid needed to lower the pH to the target pH range 5.0–5.2.

salts and acids were normalized for the small differences in tube volumes to directly compare the alkalinity production. The amount of acid neutralized in B5 agrees very well with the sum of the individual acid neutralization processes evaluated in the other tubes (Fig. 6): H^+ consumption in the HCl tube (ALK + $\Sigma\Delta$Cations), SO_4^{2-} consumption in the H_2SO_4 tube ($\Delta SO_4^{2-} = AA_{SO_4} - AA_{Cl}$), and NO_3^- consumption in the HNO_3 tube ($\Delta NO_3^- = AA_{NO_3} - AA_{Cl}$). Thus, the combination of these processes in the one tube shows no diminution or enhancement of abiotic or biotic activity when compared to individual acid tubes. The biologically mediated sedimentary processes consuming HNO_3 and H_2SO_4 and the abiotic acid neutralization processes are independent of each other at this loading rate and proceed at the same rate in the presence or absence of other strong acid anions.

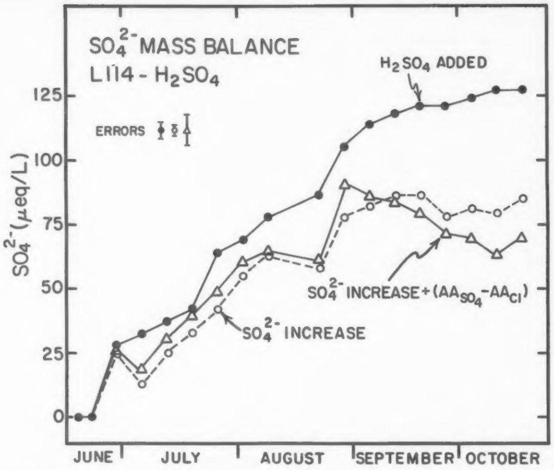

FIG. 10. Sulfate mass balance in the Lake 114 H_2SO_4 tube in 1982. Terms are the same as in Fig. 4.

6. Acid Neutralization in Lake 114: 1982

In Lake 114, the quantity of nitric acid added to the HNO_3 tube exceeds the amount of acid added to the HCl and H_2SO_4 tubes by a factor of three by midsummer (Fig. 7), indicating the important buffering effect of NO_3^- consumption. Unlike the Lake 302 tubes, the amount of HCl added is the same as the quantity of H_2SO_4 added within the uncertainty in the tube volumes. The rate of nitric acid consumption in Lake 114 was so high that the pH could not be controlled within the target pH range 5.0–5.2 with daily acid additions (Fig. 8).

In the HCl tube, the sum of the water column alkalinity plus the $\Sigma\Delta$Cations is less than the acid added by, on average, 15% (Fig. 9). Calcium, Mg^{2+}, and NH_4^+ released from the sediments are the dominant contributors to buffering in this lake (Fig. 9).

Sulfate consumption is an important term in the alkalinity production balance (Fig. 9). Lake 114 had been receiving additions of H_2SO_4 for 3 yr prior to 1982. Although the lake is not acidic, SO_4^{2-} levels have been artificially elevated. Consequently, SO_4^{2-} consumption contributes significantly to H^+ neutralization in all Lake 114 tubes.

Consumption of Cl^- also occurred in all Lake 114 tubes. Ambient lake concentrations are generally at the level of detection (<0.5 mg/L) and the precision of Cl^- analyses at these low water concentrations was poor (±10%). In the HNO_3 and H_2SO_4 tubes, the Cl^- consumption was within the analytical error. Chloride consumption was a significant contributor to total H^+ neutralization in the HCl tube (Fig. 9) where higher Cl^- concentrations were easily measured. Chloride consumption has been observed repeatedly in ELA enclosure experiments (Anderson and Schiff, unpublished data) and in whole-lake mass budgets (Schindler et al. 1986) but the processes consuming Cl^- remain unidentified.

Unlike the limnocorrals in Lake 302, the amount of acid neutralized in the H_2SO_4 tube in excess of the H^+ neutralization in the HCl tube ($AA_{SO_4} - AA_{Cl}$), plus the increase in SO_4^{2-} is less than the added H_2SO_4 (Fig. 10). This is because NH_4^+ accumulation was lower in the H_2SO_4 tube than in the HCl tube (Fig. 11). Since the term (ALK + $\Sigma\Delta$Cations) was

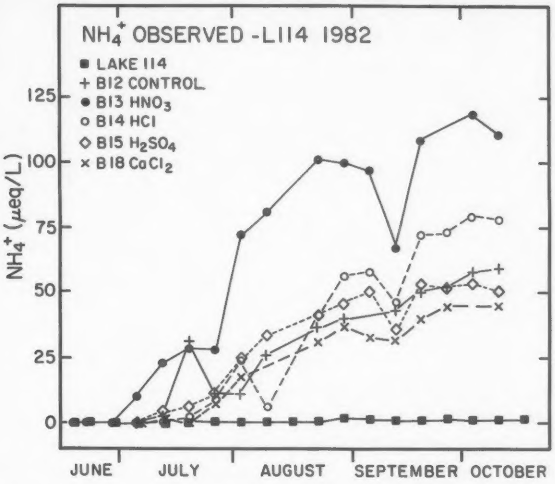

FIG. 11. Temporal pattern of observed water column NH_4^+ concentrations in the Lake 114 tubes in 1982.

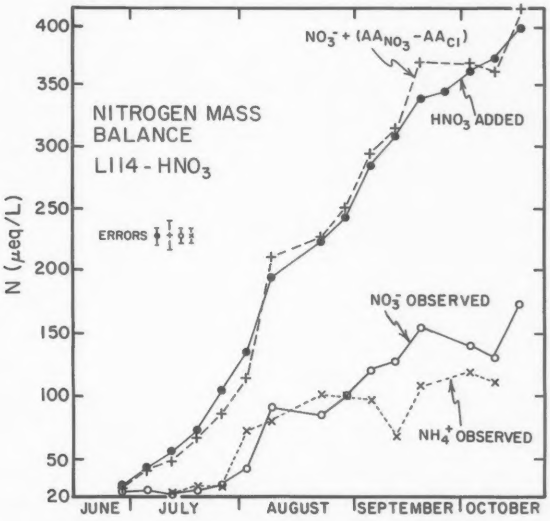

FIG. 12. Nitrate mass balance in the Lake 114 HNO_3 tube in 1982. Terms are the same as in Fig. 5. Initial water column NO_3^- concentrations was negligible.

not equal in the HCl and H_2SO_4 tubes as it was in Lake 302, the procedure used to assess the stoichiometry of the relationship between H^+ and SO_4^{2-} in Lake 302 cannot be applied to Lake 114.

HNO_3 has a much smaller potential for acidification than HCl or H_2SO_4 (Fig. 7). The NO_3^- observed is much less than the HNO_3 added (Fig. 12), indicating that most of the added NO_3^- had been consumed. Chlorophyll *a* in the HNO_3 tube showed no significant deviation from the other acid or control 7.7tubes. If the chlorophyll *a* to particulate nitrogen ratio remained constant during the experiment then the primary NO_3^- consumption process is probably denitrification rather than incorporation of nitrate into the particulate phase via photosynthesis.

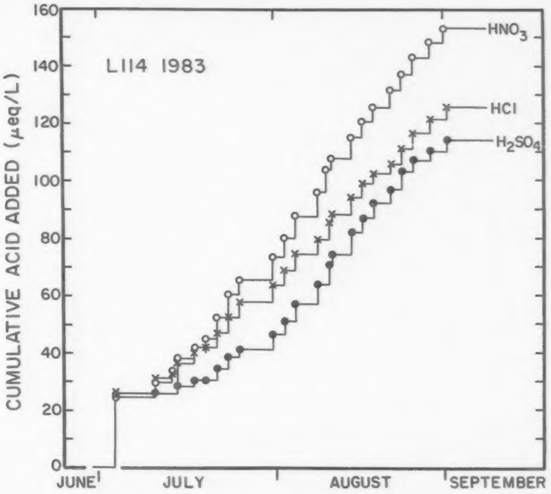

FIG. 13. Cumulative acid additions necessary to maintain a pH of 5.0 in the Lake 114 tubes in 1983.

The HNO_3 added is approximately equal to the sum of the amount of H^+ neutralized in the HNO_3 tube in excess of the H^+ neutralized in the HCl tube ($AA_{NO_3} - AA_{Cl}$) plus the NO_3^- observed (Fig. 12). However, this balance is fortuitous. NH_4^+ is elevated in the HNO_3 tube in comparison with the HCl and H_2SO_4 tubes (Fig. 11), so the $\Sigma\Delta$Cations for the HNO_3 and HCl tubes are unequal. Also, as noted earlier, the buffering effect of NO_3^- consumption in Lake 114 was so rapid that our acid addition rate was insufficient to maintain the target pH and thus consume the water column alkalinity in the HNO_3 tube (Fig. 9).

All Lake 114 tubes developed elevated NH_4^+ concentrations relative to the ambient lakewater concentration (Fig. 11). Since the flux of NH_4^+ is an important component of the acid budget in Lake 114, additional experiments were conducted in 1983 to examine the origin of these elevated NH_4^+ concentrations. Although the problems noted above prevented the assessment of the stoichiometric relationships in Table 1, the Lake 114 experiments in 1982 demonstrate the release of Ca^{2+}, Mg^{2+}, and NH_4^+ from the sediments, and dramatically emphasize the importance of NO_3^- and SO_4^{2-} consumption to acid neutralization.

7. Acid Neutralization in Lake 114: 1983

In 1983, fewer acid additions were made to a set of four tubes deployed in Lake 114, with the emphasis placed on obtaining better charge and mass balances. As in 1982, more HNO_3 than HCl or H_2SO_4 was added to the enclosures (Fig. 13). However, our acid addition rate in 1983 was insufficient to maintain the target pH in all tubes (Fig. 14), preventing inter-tube comparisons (similar to Fig. 10 and 12).

To identify the acid neutralization processes, the individual anion/cation balance for each 1983 tube was examined (Fig. 15). Overall, the sum of the calculated components of acid neutralization agrees well with the cumulative acid added for each of the three acidified tubes indicating that all the important acid neutralization processes have been identified. Release of Ca^{2+} and NH_4^+ from the sediment was the main buffering

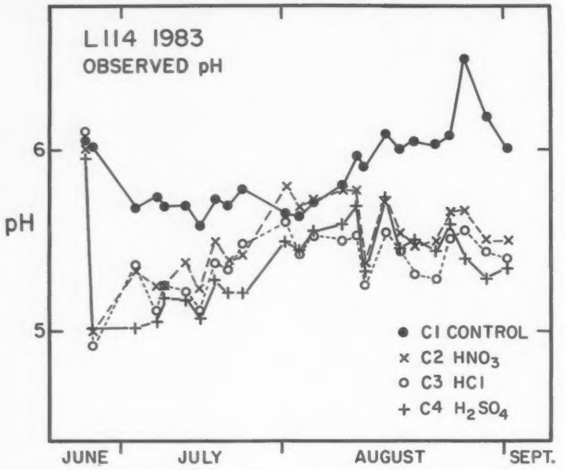

FIG. 14. Temporal pattern of observed water column pH in Lake 114 tubes in 1983.

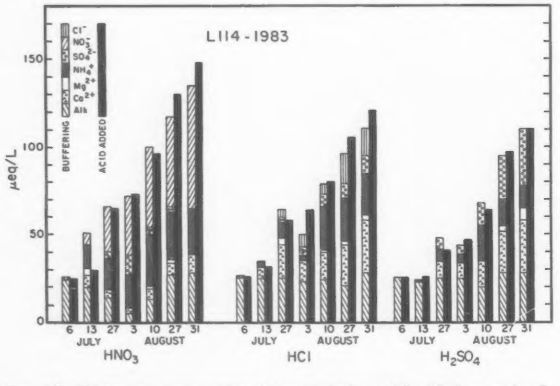

FIG. 15. Histogram contrasting the cumulative acid added to each of the acidified Lake 114 tubes in 1983 with the sum of the individual acid neutralizing processes. Individual components of buffering are calculated simply as the difference between the concentration of each ion (including alkalinity) in the control tube and in the acidified tube, except for NO_3^-, SO_4^{2-}, and Cl^- in the HNO_3, H_2SO_4, and HCl tubes, respectively. Buffering derived from NO_3^- consumption in the HNO_3 tube, SO_4^{2-} consumption in the H_2SO_4 tube and Cl^- consumption in the HCl tube is calculated from the difference between the observed NO_3^-, SO_4^{2-}, or Cl^- (after subtraction of the NO_3^-, SO_4^{2-}, or Cl^- in the control tube) and the added HNO_3, H_2SO_4, or HCl. It is assumed that the consumption ratio of H^+:NO_3^-, H^+:SO_4^{2-} and H^+:Cl^- is 1:1 (in equivalents).

mechanism in the HCl tube and was also an important source of buffering in the H_2SO_4 and HNO_3 tubes. In the HNO_3 tube, buffering was predominantly supplied by NO_3^- consumption. Since the calculated buffering agrees with the HNO_3 added (Fig. 15), the main pathway of NO_3^- disappearance must be denitrification to an uncharged end-product, either N_2 or N_2O, or incorporation as NH_3 in an organic compound.

Unlike the 1982 experiments in Lake 114, more HCl was added than H_2SO_4 in 1983 (Fig. 13). SO_4^{2-} consumption occurred in all tubes but occurred to a greater extent in the H_2SO_4 tube (Fig. 15), as observed in the Lake 114 enclosures in 1982. Addition of HCl yielded as much NH_4^+ after 3 August as the addition of HNO_3, and more NH_4^+ than addition of H_2SO_4 (Fig. 16). This greater NH_4^+ flux in the HCl tube relative to the H_2SO_4 tube exceeds the additional acid neutralized in the H_2SO_4 tube due to enhanced SO_4^{2-} consumption, and accounts for the larger acid requirement of the HCl tube. Despite the variable accumulation of NH_4^+, the enclosure experiments allow the dominant acid neutralizing processes to be identified and demonstrate that HNO_3 is neutralized at a substantially higher rate than HCl or H_2SO_4.

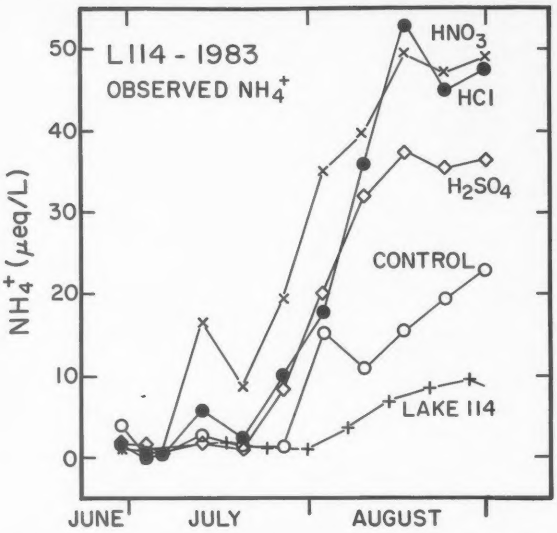

FIG. 16. Temporal pattern of observed water column NH_4^+ concentrations in Lake 114 tubes in 1983.

8. Depth of H^+ Neutralization in the Sediments

To examine pH, alkalinity, and NH_4^+ fluxes from the sediments, porewater samplers were deployed in the Lake 114 enclosures at 3 times in 1983. Porewater pH profiles collected 30 days after the initial acid addition to the tubes are shown in Fig. 17.

Assuming no neutralization of H^+, the distribution of H^+ in the sediments can be calculated according to the equation:

$$(13) \qquad (C_z - C_\infty) = (C_0 - C_\infty)\ erfc(z/2\sqrt{D_{\text{eff}}t})$$

where C_z is H^+ concentration ($[H^+]$) at depth z in the sediments at time t, D_{eff} is the effective diffusion coefficient of H^+ which can be approximated as $D^0\Phi^2$ (Lerman 1979), where D^0 is the cross-coupled molecular diffusion coefficient (2.2×10^{-5} $cm^2 \cdot s^{-1}$), Φ is the sediment porosity ($\Phi = 0.98-0.99$), and C_0 is the $[H^+]$ above the sediment–water interface. For $t = 30$ days, $C_0 = 6.3 \times 10^{-6}$ $\mu eq \cdot L^{-1}$ (pH = 5.2) and $C_\infty = 1 \times 10^{-6}$ $\mu eq \cdot L^{-1}$ (pH = 6; Fig. 17), the calculated pH porewater profile is shown by the dashed line in Fig. 17. Clearly, the added H^+ was neutralized within the upper 1–2 cm of the sediments, much shallower than the predicted penetration in the absence of neutralization, and the porewater $[H^+]$ profile could have been approaching a steady-state distribution by day 30.

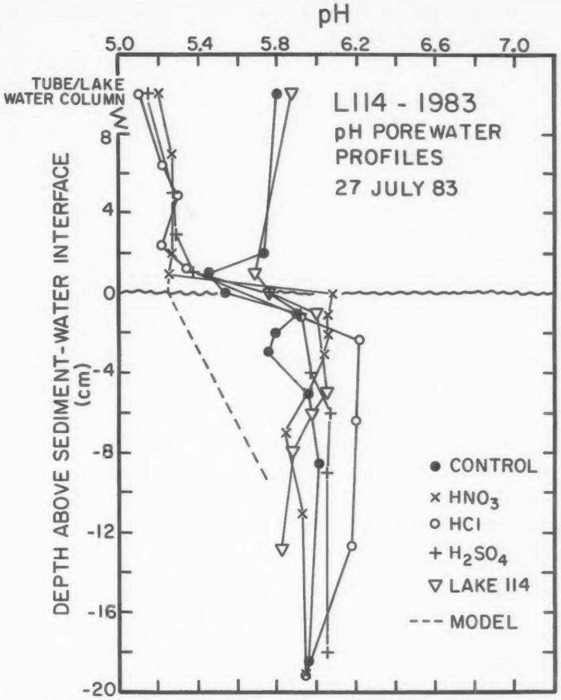

FIG. 17. Profiles of porewater pH in the sediments of Lake 114 and Lake 114 tubes in 1983. The dashed line represents the calculated penetration of H^+ into the sediments by molecular diffusion after 30 days in the absence of any neutralization.

9. Origin of Elevated NH_4^+ Concentrations in Lake 114 Enclosures

Water column NH_4^+ concentrations were elevated in the Lake 114 tubes above the ambient lake concentrations in 1981 (data not shown), 1982 (Fig. 11), and 1983 (Fig. 16). Accumulation of NH_4^+ was observed in the control tubes as well as the acidified tubes, and thus is not entirely an effect of the addition of acid, but appears to be, at least in part, an artifact of the enclosure technique. At the end of the 2-month period in 1983, the ratio of the accumulated NH_4^+ concentration in the control tube to the NH_4^+ concentration in Lake 114 is approximately 2 (Fig. 16). The ratio of the tube depth to the mean lake depth is also close to 2, and if the NH_4^+ flux was uniform everywhere in the lake, then the elevated NH_4^+ concentrations observed in the control tube in 1983 may simply reflect the shallower mean depth of the enclosures relative to the lake.

Porewater NH_4^+ profiles in the lake and in the tubes 5 wk after emplacement of the enclosures in 1983 are shown in Fig. 18. No systematic difference is evident between the control tube and the lake between 0–5 cm, indicating that the NH_4^+ flux from within the bulk sediment is similar in the lake and in the tube experiments. Thus, the NH_4^+ flux from the sediments is always high, and the enclosed water column in the tubes must have been cut off from the primary NH_4^+ sink for the lake. Applying Fick's first law to the NH_4^+ gradient over 0–2 cm (Fig. 18) yields a flux of 8.6×10^{-7} $\mu eq \cdot cm^{-2} \cdot s^{-1}$.

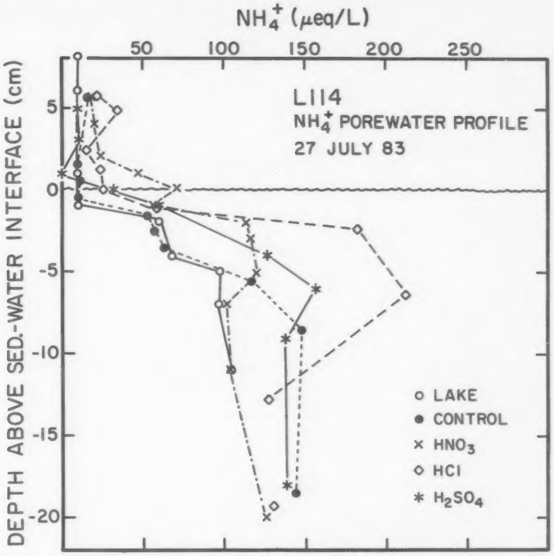

FIG. 18. Profiles of porewater NH_4^+ in the sediments of Lake 114 and Lake 114 tubes in 1983.

Accumulation of this NH_4^+ flux in the tubes over a 2 month period would result in water column NH_4^+ concentrations of 37 $\mu eq \cdot L^{-1}$, very close to the measured NH_4^+ accumulation (Fig. 16). Ammonia released from diagenesis of organic matter is not rapidly consumed by nitrifiers. Nitrification is naturally a slow process in ELA lakes and proceeds at an undetectable rate during summer stratification (J.W.M. Rudd, Freshwater Institute, Winnipeg, pers. comm. 1984). Elevated NH_4^+ concentrations in the enclosures must result from either an inhibition of phytoplankton or periphyton assimilation of NH_4^+ in the water column, enhanced decomposition at the sediment–water interface in the tubes relative to the lake, or the exclusion of another natural NH_4^+ sink for the lake such as the bog vegetation which surrounds the lake.

Ammonia concentrations are elevated in the acidified tubes relative to the control tubes. The effect was more noticeable in 1981 (data not shown) and 1983 (Fig. 16) than in 1982 (Fig. 11). Ammonia is adsorbed to the sediments of Lake 114 and could ion-exchange with the added H^+ (Table 4) in the upper 2 cm of the sediments where the acid is neutralized (Fig. 17). The inventory of exchangeable NH_4^+ in the upper 2 cm is 0.22 $\mu eq \cdot cm^{-2}$, corresponding to a maximum accumulation of only 2 $\mu eq \cdot L^{-1}$ of NH_4^+ in the tube water column if entirely displaced by H^+. Additional support that NH_4^+ does not accumulate as a result of ion-exchange with H^+ is supplied by the $CaCl_2$ experiment, where exchange of NH_4^+ for Ca^{2+} was not detected (Fig. 11). Therefore, the additional NH_4^+ accumulation in the acidified tubes could not be supplied by ion-exchange with H^+ but must have been derived from the continuous degradation of organic matter coupled with inhibition of NH_4^+ consumption. Phytoplankton or periphyton assimilation of NH_4^+ may have been inhibited by the addition of acid, but the inventory of organic nitrogen (2.4% by weight; Brunskill et al. 1971) in the upper 10 cm of the sediment column (750 $\mu eqN \cdot cm^{-2}$) is sufficient to allow the flux of remineralized NH_4^+ out of the sediments to continue uninter-

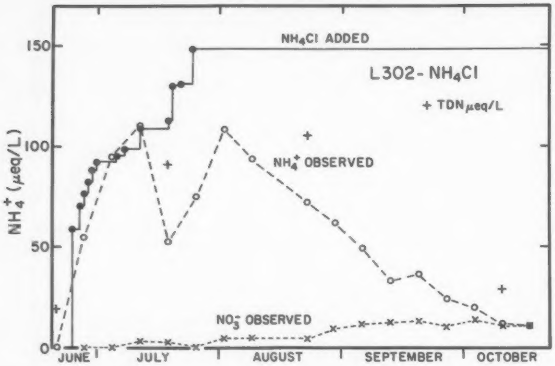

FIG. 19. Nitrogen mass balance in the NH_4Cl tube in Lake 302. "NH_4Cl added" is the cumulative NH_4Cl added to the tube.

rupted for several years.

Water column NH_4^+ concentrations in the HNO_3 tubes in 1981, 1982, and 1983 (before 3 August) were further elevated in comparison with the other acidified and control tubes. Two possibilities may account for these elevated NH_4^+ concentrations; (1) increases sedimentation and decomposition of organic matter due to addition of NO_3^- as nutrient, or (2) denitrification of NO_3^- to NH_4^+. These two possibilities cannot be distinguished on the basis of water column data alone. Either possibility will result in the same net acid neutralization with one μeq H^+ consumed per μeq NO_3^- consumed.

Since NH_4^+ is an important component of acid neutralization in Lake 114, changes in the dynamics of NH_4^+ recycling due to increased H^+ or toxic metal concentrations will affect the lake alkalinity budget.

10. Acidifying Effect of NH_4^+: Lakes 302 and 114

In one Lake 302 tube, NH_4^+ added as NH_4Cl was gradually removed from the water column during the course of the experiment (Fig. 19). The TDN analyses track the NH_4^+ disappearance indicating loss of dissolved nitrogen from the water column. The chlorophyll *a* analyses do not reflect any increase in algal biomass and thus storage of nitrogen in particulate form in the water column. The NH_4^+ must be removed by nitrification, cation exchange, periphyton uptake, or assimilation by rapidly settling phytoplankton. The small quantity of NO_3^- released to the water column (Fig. 19) indicates that a small percentage of the nitrification proceeds to NO_3^-.

The observed pH in the Lake 302 NH_4Cl tube dropped to the same level as the HCl, HNO_3 and H_2SO_4 tubes in September (Fig. 2). The loss of NH_4^+ by mid-September was 120 $\mu eq \cdot L^{-1}$ (Fig. 19) comparable to the acid added to the HCl tube (117 $\mu eq \cdot L^{-1}$) during the same interval. Ammonia must have been removed from the water column as uncharged NH_3 leaving behind HCl which was subsequently consumed by similar processes as in the HCl tube. Because ion exchange of NH_4^+ with basic cations in the sediments will remove NH_4^+ from the water column but will not contribute to acidity production, the major mechanism of NH_4^+ removal must have been either nitrification to a gaseous end-product or sequestering of NH_4^+ as organic nitrogen. Thus, alkalinity is consumed by the addition of an ammonium salt to a natural lake system (see also Schindler et al. 1985b).

The interpretation of the results from the Lake 114 NH_4Cl tube is complicated by the natural flux of NH_4^+ from the sediments so the result of NH_4Cl addition to the Lake 114 tube could not be assessed.

11. Exchange Effects of Ca^{2+}: Lakes 302 and 114

$CaCl_2$ was added to the experimental tubes at a Ca^{2+} loading rate equivalent to lime additions of acidified lakes in Sweden (Bengtsson et al. 1980). Increasing the Ca^{2+} concentrations from 1 to 5 ppm resulted in an increase in major cations, particularly Mg^{2+} (by approximately 30%) in both Lakes 114 and 302 (data not shown).

A pH decrease (H^+ increase) was also noted in Lake 114 (Fig. 8). This effect was reproduced by addition of $CaCl_2$ in September to one control tube in Lake 114 and by a second addition of $CaCl_2$ in the Lake 114 $CaCl_2$ tube, resulting in a comparable decrease in pH (Fig. 8). A corresponding decrease in the observed pH in the Lake 302 $CaCl_2$ tube was not observed (Fig. 2). Due to the higher water column alkalinity of Lake 302, release of H^+ in Lake 302 equal to the release of H^+ in Lake 114 as a consequence of $CaCl_2$ addition would be insufficient to produce a measureable pH decrease in the Lake 302 $CaCl_2$ tube.

Cation exchange of Ca^{2+} and H^+ has important implications for the addition of lime as a mitigation strategy for acid-stressed lakes. The alkalinity demand of the sediments will be a result of both the acidity of the surficial sediments and the effect of cation exchange between H^+ and Ca^{2+}. Although the alkalinity demand of sediments in acidic lakes has been documented (e.g. Yan and Dillon 1984), these experiments show that the alkalinity demand of non-acidic lakes is also appreciable. The addition of a neutralizing agent with a lower ion-exchange affinity (e.g. Na^+, see also Table 4) would reduce the sediment alkalinity demand.

NH_4^+ (Fig. 11) and the trace metals: Zn, Al, and Mn (data not shown), were not significantly mobilized from the sediments by the Ca^{2+} additions.

12. Comparison of HCl, HNO_3, and H_2SO_4 acid neutralization rates

Replication between 1982 and 1983 is reasonable in the Lake 114 HCl and H_2SO_4 enclosures (Table 5). Less HNO_3 was added in 1983 than in 1982 (Table 5). Since the target pH was not maintained in Lake 114 in either year, the capacity of the Lake 114 sediments to neutralize HNO_3 at pH 5.0 was substantially underestimated in both years. The quantity of HNO_3 neutralized in excess of the water column alkalinity exceeded the amount of HCl neutralized by a factor of 3 at the end of 2 months in both Lake 114 in 1983 and Lake 302 (Table 5). If the target pH of 5.0–5.2 had been maintained in Lake 114, the difference between the acid neutralized in the HNO_3 and HCl tubes would have been even more striking.

The quantity of HNO_3 added exceeded the quantity of H_2SO_4 added in both lakes in 1982 (Table 5). Nitrate consumption per unit sediment area occurred at a rate five times faster than sulfate consumption (Table 5). HNO_3 thus has a much smaller potential for acidification than H_2SO_4.

The rate of SO_4^{2-} consumption increased in response to an increase in SO_4^{2-} loading observed in Lake 114 in 1982 and 1983 (sections 6 and 7) indicating that SO_4^{2-} reducing bacteria are SO_4^{2-} limited. The rapid rate of HNO_3 neutralization also

TABLE 5. Comparison of acid added, denitrification, SO_4^{2-} consumption and inorganic sediment buffering in Lakes 302 and 114.

	Lake 302	Lake 114:1982	Lake 114:1983
Initial alkalinity ($\mu eq \cdot L^{-1}$)	77	30	25
Acid added after 2 months to open-bottom tubes: ($\mu eq \cdot L^{-1}$)			
HCl:	114	110	122
H_2SO_4:	137	114	110
HNO_3:	206	285	143
Acid consumption in excess of initial alkalinity after 2 months: ($\mu eq \cdot cm^{-2} \cdot 2\ months^{-1}$)			
HCl:	3.9	10.0	11.7
H_2SO_4:	5.8	11.1	10.5
HNO_3:	13.8	34.4	14.5
Nitrate consumption[a]: ($\mu eq \cdot cm^{-2} \cdot 2\ months^{-1}$)	11.4	22	9.5
Sulfate consumption[b]: ($\mu eq \cdot cm^{-2} \cdot 2\ months^{-1}$)	2.2	4.2	3.9
Inorganic sediment buffering[c]: ($\mu eq \cdot cm^{-2} \cdot 2\ months^{-1}$)	3.9	10.0	11.7
[HNO_3 consumption — HCl consumption]/ denitrification:	0.9	1.1	0.3
[H_2SO_4 consumption — HCl consumption]/ sulfate consumption:	0.9	0.3	−0.3

[a] Calculated from disappearance of water column NO_3^- in the HNO_3 tube.
[b] Calculated from disappearance of water column SO_4^{2-} in the H_2SO_4 tube.
[c] Calculated from HCl added to maintain the target pH in the HCl tube.

suggests that denitrifiers are NO_3^- limited. If the rates of NO_3^- and SO_4^{2-} consumption in the sediments are both limited by diffusion into the sediments, then the relative depths at which the two processes occur can be calculated from Fick's first law:

$$F = D_{\mathrm{eff}} \frac{\delta C}{\delta z} \tag{14}$$

where F is the flux (Table 5), D_{eff} is the effective diffusion coefficient (Lerman 1979), and δC is the difference between the water column concentration and the porewater concentration below the zone of NO_3^- and SO_4^{2-} consumption (Kelly and Rudd 1984). Rearranging equation (14) and inserting the fluxes from Table 5 yields a ratio of the depth of SO_4^{2-} consumption (δz_{SO_4}) to the depth of NO_3^- consumption (δz_{NO_3}) of 4.8 in Lake 302 and 5.0 in Lake 114. These results are consistent with measured porewater profiles which show that $[NO_3^-]$ approaches 0 by the first sample (generally 1 cm), whereas $[SO_4^{2-}]$ decreases linearly over the first few centimetres (Kelly and Rudd 1984; Schiff and Anderson 1986). Furthermore, the pH gradient in the HNO_3 tube is the sharpest of the three acidified tubes (Fig. 17) consistent with the conclusion that HNO_3 is neutralized by NO_3^- reduction very close to the sediment–water interface, whereas SO_4^{2-} consumption occurs well below the zone of NO_3^- consumption. Consequently, our observation that HNO_3 is less efficient at acidifying lakes than H_2SO_4 may not reflect a greater efficiency or rate of NO_3^- reduction, but that diffusive transport is rate limiting for both processes. Neutralization of HNO_3 is more rapid because NO_3^- reduction occurs at shallower depths in the sediments than SO_4^{2-} consumption.

13. Comparison Between Lake 114 and Lake 302 Acid Neutralization Rates

Lake 302 and Lake 114 differ in the rate of acid neutralization. To maintain the target pH, the amount of HCl added in excess of the initial alkalinity in Lake 114 was more than twice the amount in Lake 302 (Table 5), even though Lake 302 had a much higher initial alkalinity than Lake 114. Thus, ambient water column alkalinity is not a good predictor of the short-term acid buffering capacity of the lake sediments and alkalinity surveys of large numbers of lakes may not accurately reflect the acid neutralization capacity of the sediments.

After the first few weeks of acid additions, the rate of HCl consumption decreased in the Lake 302 HCl tube and the pH stabilized (Fig. 1). The HCl consumption plateau in Lake 302 indicates that the readily accessible abiotic acid buffer capacity was rapidly depleted. In Lake 114 , the rate of HCl neutralization did not decrease sharply as in Lake 302 (Fig. 6). The lower rate of HCl consumption in Lake 114 in September and October may have been due to a decrease in either the accessible buffer capacity or the ambient lake temperature.

The processes responsible for the enhanced acid neutralization in Lake 114 over Lake 302 can be assessed by comparing the HCl buffering balances for Lake 302 (Fig. 3) and Lake 114 in 1982 (Fig. 9) and 1983 (Fig. 15). A comparable amount of Ca^{2+} was released to the water column after the initial 2-month period in Lake 114 and in Lake 302. The concentration of exchangeable cations in $\mu eq \cdot cm^{-3}$ is approximately equal for all cations (except NH_4^+ and Mn^{2+}), in both the organic-rich sediments of Lake 114 and the sandy sediments of Lake 302

(Table 4). Sulfate consumption supplied some excess alkalinity (1.0 $\mu eq \cdot cm^{-2} \cdot 2$ months^{-1} or 8 $\mu eq \cdot L^{-1}$ in 1982) in Lake 114 tubes compared to Lake 302 tubes due to higher ambient SO_4^{2-} concentrations in Lake 114. The major difference between the Lake 114 and Lake 302 HCl tubes was the NH_4^+ release from the sediments. NH_4^+ contributed as much to acid neutralization as Ca^{2+} in Lake 114 but was unimportant in the Lake 302 tubes. The laboratory determined concentration of exchangeable NH_4^+ in both 302 and 114 is an order of magnitude lower than the concentration of exchangeable Ca^{2+} (Table 4). Accumulation of NH_4^+ in the Lake 114 tubes was not a simple effect of ion-exchange for added H^+ (section 9), rather NH_4^+ consumption was reduced in the enclosures as a consequence of installing the tubes.

Consumption rates of SO_4^{2-} and NO_3^- are faster in the Lake 114 than in Lake 302 by about a factor of two (Table 5), even though both sediments are anaerobic within a few centimetres of the sediment–water interface (Kelly and Rudd 1984). This observation is consistent with the hypothesis that the rates of SO_4^{2-} and NO_3^- consumption, and hence rates of acid neutralization, are limited by the diffusive flux of these species into the sediments. The much greater porosity of Lake 114 surface sediments (≈ 0.98) than in Lake 302 (≈ 0.70) would permit a much faster flux of SO_4^{2-} and NO_3^- into the sediments of Lake 114 at similar water column concentrations since the flux is proportional to Φ^3 (Lerman 1979). The ratio of $\Phi^3 (114)/\Phi^3 (302)$ for surficial sediments is 2.7, close to the ratio between the two lakes of the NO_3^- to SO_4^{2-} consumption rates. Any process which accelerates the transport of SO_4^{2-} and NO_3^- into the sediments, such as bioturbation or physical mixing of the sediments, would consequently increase the rate of acid neutralization.

14. Importance and Limits of Shallow Sediment Buffering

Of the two lakes, Lake 302 is perhaps more typical of small Canadian Shield lakes. However, many lakes have shallow bays or epilimnetic areas with sediments similar to Lake 114. The potential buffer capacity in shallow sediments is important, since during summer stratification, non-epilimnetic sediments are excluded from contact with the acid input. In many small lakes similar to Lake 302, almost 50% of the sediments lie above the seasonal thermocline.

The importance of the shallow sediment buffer capacity can be illustrated by comparing acid neutralization rates in the enclosure experiments (Table 5) with the acid load in northeastern U.S. Driscoll and Likens (1982) estimate the average hydrogen ion loading in wet and dry deposition in NE U.S. to be 13.2 $\mu eq \cdot cm^{-2} \cdot yr^{-1}$ or 2.2 $\mu eq \cdot cm^{-2} \cdot 2$ months^{-1}. The Lake 114 enclosure experiments clearly demonstrate that Lake 114 is capable of neutralizing acid input at this rate (Table 5). In the Lake 114 whole-lake acidification experiment, the lake has received a loading of 1.0 $\mu eq \cdot cm^{-2} \cdot 2$ months^{-1} (Schindler 1985) and has maintained a positive water column alkalinity since acidification began in 1979. Although Lake 302 is also capable of buffering this rate of acid loading initially, the Lake 302 acid consumption rate exhibits a marked decrease after the first month of acidification, indicating that the easily accessible non-biological buffer capacity had been depleted. The limit to the biotic and abiotic buffering supplies in lake sediments is an important variable in the reconstruction and prediction of the temporal trends of lake acidification.

Conclusions

Once the water column alkalinity is depleted, the within-lake alkalinity production processes are confined to the lake sediments. Both Lake 114, which had been receiving H_2SO_4 additions equivalent to an annual precipitation load of pH 4.0 for 3 yr prior to our experiments, and Lake 302 have significant acid buffering capacity in the sediments. Although Lake 114 has a much lower ambient water column alkalinity than Lake 302, the organic-rich flocculent sediments of Lake 114 neutralize acid at a significantly higher rate than the sandy sediments of Lake 302. Ambient water column alkalinity is not a good predictor of acid neutralization capacity in the sediments.

After the first few weeks of acid additions, the rate of HCl consumption decreased in the Lake 302 tube, indicating that the readily accessible abiotic acid buffer capacity was rapidly depleted. In Lake 114, the rate of HCl consumption did not decrease, due to the accumulation of NH_4^+ in the water column and the higher porosity of the sediments.

The principal source of non-anion-specific buffering in the enclosure experiments was Ca^{2+} release in Lake 302 and Ca^{2+} and NH_4^+ release in Lake 114. There is no significant difference between the organic-rich sediments of Lake 114 and the sandy sediments of Lake 302 in the concentration of exchangeable cations ($\mu eq \cdot cm^{-3}$) for all cations except NH_4^+ and Mn^{2+}. Since NH_4^+ is an important component of acid neutralization in Lake 114, changes in the dynamics of NH_4^+ recycling due to increased H^+ or toxic metal concentrations will affect the lake alkalinity budget.

In both Lake 302 and Lake 114, HNO_3 has a much smaller potential for acidification than HCl or H_2SO_4. Denitrification to an uncharged end-product was the main mechanism of NO_3^- removal. Sulfate consumption in the sediments also contributes to alkalinity production but at a much slower rate than denitrification. SO_4^{2-} and NO_3^- consumption rates increased in response to increased water column concentration suggesting that the consumption rates of these ions are limited by diffusion into the sediments. Slower rates of SO_4^{2-} consumption when $[SO_4^{2-}]$ and $[NO_3^-]$ are similar in the water column reflect the consumption of SO_4^{2-} at greater depth in the sediment than NO_3^- consumption. Consequently, any process which accelerates the transport of SO_4^{2-} and NO_3^- into the sediments, such as bioturbation, irrigation, or physical mixing of the sediments, would increase the rate of acid neutralization.

The acid neutralization rates by non-anion-specific buffering, nitrate consumption and sulfate consumption are independent of each other and therefore additive.

Addition of an ammonium salt results in consumption of water column alkalinity, a least in Lake 302, because NH_4^+ is biologically consumed as an uncharged species leaving behind an equivalent quanitity of H^+.

Addition of Ca^{2+} as $CaCl_2$, at a loading rate equivalent to liming of an acidified lake, perturbed the cation exchange equilibrium of the sediments, releasing Mg^{2+} in both lakes and H^+ in Lake 114.

Alkalinity production in epilimnetic sediments is an important component of the resistance of softwater lakes to anthropogenic acidification.

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Alkalinity Generation and the Fate of Sulfur in Lake Sediments

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Recent studies of major ion budgets of lakes have demonstrated the occurence of within-lake alkalinity generation, or acid consumption, that may represent a significant source of buffering against acid precipitation. Consumption of sulfate is associated with a major portion of alkalinity generation. Sources of alkalinity, and the capacities of lakes to neutralize sulfuric acid, corresponding to each of the probable fates of sulfur have not been discussed explicitly in earlier papers. This note was written to clarify these points.

De récentes études des bilans des principaux ions dans les lacs ont montré l'existence d'une génération d'alcalinité, ou d'une neutralisation des acides, au sein des lacs qui peut être importante pour la neutralisation des précipitations acides. L'utilisation des sulfates est liée à une partie importante de la génération d'alcalinité. Les sources d'alcalinité et le pouvoir des lacs à neutraliser l'acide sulfurique, qui correspondent à chacun des devenirs probables du soufre, non pat été traités se façon explicite dans les communications précédentes. La présente note a pour but d'éclaircir ces points.

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The microbial utilization of NO_3^-, MnO_2, FeOOH, and SO_4^{-2} as terminal electron acceptors for the oxidation of organic matter is known to result in the production of alkalinity (Froelich et al. 1979; Emerson et al. 1980). This fact has been incorporated into a number of discussions of the alkalinity budgets of lakes in relation to the problem of lake acidification by acid precipitation (e.g. Dickson 1980; Schindler et al. 1980; Kelly et al. 1982; Cook and Schindler 1983; Cook et al. 1986; Schindler et al. 1986). Sources of alkalinity to lake water were identified in these studies using the definition of alkalinity based on the principle of electroneutrality:

$$(1)\quad \mathrm{Alk} = Ca^{2+} + Mg^{2+} + Na^{+} + K^{+} + \text{other base cations} - SO_4^{2-} - NO_3^{-} - Cl^{-} - \text{other strong acid anions}$$

where the concentration of each ion is in $eq \cdot L^{-1}$. The usefulness of this equation can be illustrated as follows. For example, it is evident that the loss from the water column of 1 equivalent of sulfate must be balanced by one of the following: by the equivalent loss from the water column of a strong base cation, by replacement of SO_4^{2-} in the water column by an equivalent amount of another strong acid anion, by an equivalent increase in alkalinity of the water column, or by a combination of the above. This is true regardless of the fate of the lost sulfate, as will be evident from examination of individual processes below. Each major ion in lake water can be examined individually to identify sources of alkalinity, including sulfate consumption, denitrification, cation exchange, and mineral weathering. In most cases, these processes occur within the sediments, where proper collection and preservation of samples without perturbing their chemical composition (e.g. through oxidation) is difficult. In contrast, concentrations of each of these ions in lake water are easily subjected to routine analysis, an important advantage of this approach to the study of alkalinity production.

Multiple processes which produce or consume these ions occur simultaneously within the sediments. Analysis of water column samples alone cannot distinguish between these processes. Although the ΔALK:ΔION ratio in the water column may be the same for different sedimentary processes, the actual source of the alkalinity and the long-term capacity of the system to produce alkalinity may be very different for each process. This is particularly true for sulfate. The ion balance approach has been used to identify the important role of sulfate consumption in within-lake acid neutralization (Kelly et al. 1982; Cook and Schindler 1983; Cook et al. 1986; Schindler et al. 1986; Schiff and Anderson 1987a). However, the fate of the sulfur, the actual source of the alkalinity replacing SO_4^{2-} lost from the water column, and consequently the long-term capacity for lakes to neutralize H_2SO_4, was not fully considered in these references.

Although iron and sulfur cycles are closely coupled, it is important to identify alkalinity generated from reduction of iron and from consumption of sulfur separately in order to extrapolate to end member systems in which either Fe(III) or SO_4^{-2} is limiting or effectively absent. It is also important to define the limiting component of each acid neutralizing process to determine the ultimate capacity of the system to neutralize acid. It is assumed in deriving these relationships that all of the reactive Fe in the sediments is derived from weathered iron oxyhydroxides initially formed in the watershed and supplied to the lake by erosion from the watershed. Fe bound within aluminosilicate matrices, whether as Fe(II) or Fe(III), is assumed to be non-reactive.

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Reduction of NO_3^- and of MnO_2 are not considered here because the contribution to alkalinity by these reactions has been clearly explained in the literature (e.g., Froelich et al. 1979; Emerson et al. 1980). The presence of $CaCO_3$ in sediments would completely alter the stoichiometries of alkalinity producing or consuming reactions listed below through the subsequent reaction of the metabolic products with $CaCO_3$ (Froelich et al. 1979; Emerson et al. 1980). Therefore, the following reactions are written for lakes with $CaCO_3$-free sediments and pore waters which are undersaturated with respect to $CaCO_3$. One reason for interest in these alkalinity-producing reactions is to identify their potential capacity to neutralize acid precipitation. It is appropriate to consider these reactions under these conditions because lakes which are most sensitive to acid precipitation have little or no $CaCO_3$ in their watersheds or sediments.

If the reduced products, Fe^{+2} and H_2S, combine to form FeS:

$$Fe^{+2} + H_2S \rightarrow FeS + 2\ H^+ \tag{8}$$

2 protons are produced, so that when sulfate is reduced in the presence of pre-existing Fe^{2+}:

$$2\ CH_2O + SO_4^{-2} + Fe^{+2} \rightarrow 2\ CO_2 + FeS + 2\ H_2O \tag{9}$$

there is no net production or consumption of protons. There would similarly be no consumption of protons following sulfate reduction and precipitation of sulfide with any other metal which occurs only as a free cation and does not consume H^+ during its transformation into a sulfide.

Combining reactions (2) and (9) provides the net effect resulting from Fe(III) reduction, sulfate reduction, and burial of FeS:

$$9CH_2O + 4\ FeOOH + 4\ SO_4^{-2} + 8\ H^+ \rightarrow 9CO_2 + 4\ FeS + 15\ H_2O \tag{10}$$

where 8 protons have been consumed, the same as for reaction (2), although 9 times as much organic matter has been oxidized. The ratio of H^+ consumed: SO_4^{2-} consumed is 1:1 (eq:eq), although the only net H^+ consuming process in the sequence of reactions combined to form equation (10) is the reduction of Fe(III) and the conversion of one solid form of Fe (FeOOH) to another (FeS) in the sediments. In terms of ions lost from solution, and therefore measurable by a water column mass budget, SO_4^{2-} loss has been accompanied by an equivalent loss of H^+ (gain of alkalinity). However, this sequence of proccesses does not represent a net production of new alkalinity in the combined water + sediment system, but rather represents a net transfer of sedimentary Fe−ANC to the water column, and a permanent depletion of the sedimentary Fe−ANC.

Inorganic sulfur may, however, be buried in forms other than FeS. For example, elemental sulfur must form, at least temporarily, in lake sediments:

$$SO_4^{-2} + 2\ H^+ + 3\ H_2S \rightarrow 4\ S° + 4\ H_2O \tag{11}$$

because pyrite, which forms by the reaction (Berner 1984):

$$FeS + S° \rightarrow FeS_2 \tag{12}$$

In the following discussion a simplified form of organic matter, CH_2O, is used. Although the release of organic nitrogen is not negligible in the overall alkalinity budgets of these reactions, we are concerned here only with the production and consumption of alkalinity derived from the chemical transformations of iron and sulfur. Readers can find information on the organic nitrogen contribution to the alkalinity budgets in Froelich et al. (1979) and Schiff and Anderson (1987a) for example.

Iron Reactions

The stoichiometric relationship for Fe(III) reduction during the microbial utilization of organic matter can be written as:

$$CH_2O + 4\ FeOOH + 8\ H^+ \rightarrow CO_2 + 4\ Fe^{+2} + 7\ H_2O \tag{2}$$

Eight equivalents of protons are consumed during the oxidation of one mole of organic carbon as represented by this reaction. Equation (2) could be combined with the equilibrium relationships for the hydrolysis and dissociation of CO_2:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \tag{3}$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \tag{4}$$

and rewritten as:

$$CH_2O + 4\ FeOOH + CO_2 + H_2O \rightarrow 8\ HCO_3^- + 4\ Fe^{+2} \tag{2'}$$

When production of bicarbonate ion is included in the reaction sequence, it becomes clear that the consumption of H^+ is chemically equivalent to the production of alkalinity and vice versa.

In a seasonally stratified lake with an anoxic hypolimnion, the release of Fe^{+2} to the anoxic water column is accompanied by a build-up of alkalinity (e.g. Kelly et al. 1982; Cook et al. 1986) according to equations (1) and (2), while mixing at overturn causes dissolved Fe^{+2} to react with O_2 with the concomitant consumption of alkalinity:

$$4Fe^{+2} + O_2 + 6\ H_2O \rightarrow 4\ FeOOH + 8\ H^+ \tag{5}$$

In the end member situation in which there is no process which could sequester Fe(II), all of the Fe^{+2} that is released to the anoxic water column during stratification is oxidized at overturn. Since the ratio of (H^+ produced)/(Fe oxidized) in Equation (5) is identical to the ratio of (H^+consumed)/(Fe reduced) in Equation (2), there would be no net production or consumption of alkalinity on an annual basis.

A similar Fe(III)–Fe(II) cycling occurs within sediments underlying an oxygenated water column. Respiration depletes oxygen to undetectable levels a few mm to a few cm below the sediment–water interface (Kelly and Rudd 1984). Below the depth of O_2 penetration, microbial respiration begins to reduce FeOOH to Fe^{2+}(eq. 2), causing dissolved $Fe^{2+}$to accumulate in pore waters (Kuivila and Murray 1984; Cook et al. 1987b; Schiff and Anderson 1987b). Dissolved Fe^{2+}diffuses upward according to Fick's first law until it encounters O_2 where it is oxidized and precipitates again as FeOOH (eq. 5). These redox and upward transport processes occur entirely within the sediments underlying an oxygenated water column, maintaining a high concentration of solid FeOOH near the sediment–water interface.

If Fe^{2+} does not react further to consume alkalinity through precipitation of $FeCO_3$, then the rate of production of alkalinity which may be transferred to the water column is equal to the rate at which Fe is removed from the FeOOH $\leftrightarrow Fe^{2+}$ cycle and permanently buried as Fe(II), for example as FeS and FeS_2. The inventory of reducible FeOOH in sediments underlying an oxygenated water column represents a potential source of permanent acid neutralization if coupled to a process that permanently buries Fe as Fe(II). FeOOH can be thought of as a "solid phase alkalinity" or acid neutralization capacity which will be referred to below as Fe–ANC.

Inorganic Sulfur Reactions

The chemical reaction for sulfate reduction can be written as:

(6) $$2\,CH_2O + SO_4^{-2} + 2\,H^+ \rightarrow 2\,CO_2 + H_2S + 2\,H_2O$$

where H^+ is consumed on an equivalent basis with SO_4^{2-}. However, if H_2S is reoxidized to SO_4^{2-}:

(7) $$H_2S + 2\,O_2 \rightarrow SO_4^{-2} + 2\,H^+$$

the chemical redox cycle is closed. Two protons are produced and there is no net change in alkalinity, similar to the case for a closed iron cycle.

Microbial reduction of sulfate is ubiquitous in lake sediments within a few cm of the sediment–water interface, even in sandy littoral sediments (Kuivila and Murray 1984; Kelly and Rudd 1984; Schiff and Anderson 1987b; Rudd et al. 1986a). In Lake 223 at the Experimental Lakes Area, during 8 years for which an accurate sulfate budget could be constructed, more SO_4^{2-} was reduced in the sandy littoral sediments than in the anoxic hypolimnion (Cook et al. 1986). Therefore, the processes described here apply to all lakes, not only those with seasonally anoxic hypolimnia.

If the reduced products, Fe^{+2} and H_2S, combine to form FeS:

(8) $$Fe^{+2} + H_2S \rightarrow FeS + 2\,H^+$$

2 protons are produced, so that when sulfate is reduced in the presence of pre-existing Fe^{2+}:

(9) $$2\,CH_2O + SO_4^{-2} + Fe^{+2} \rightarrow 2\,CO_2 + FeS + 2\,H_2O$$

there is no net production or consumption of protons. There would similarly be no consumption of protons following sulfate reduction and precipitation of sulfide with any other metal which occurs only as a free cation and does not consume H^+ during its transformation into a sulfide.

Combining reactions (2) and (9) provides the net effect resulting from Fe(III) reduction, sulfate reduction, and burial of FeS:

(10) $$9CH_2O + 4\,FeOOH + 4\,SO_4^{-2} + 8\,H^+ \rightarrow 9CO_2 + 4\,FeS + 15\,H_2O$$

where 8 protons have been consumed, the same as for reaction (2), although 9 times as much organic matter has been oxidized. The ratio of H^+ consumed: SO_4^{2-} consumed is 1:1 (eq:eq), although the only net H^+ consuming process in the sequence of reactions combined to form equation (10) is the reduction of Fe(III) and the conversion of one solid form of Fe (FeOOH) to another (FeS) in the sediments. In terms of ions lost from solution, and therefore measurable by a water column mass budget, SO_4^{2-} loss has been accompanied by an equivalent loss of H^+ (gain of alkalinity). However, this sequence of proccesses does not represent a net production of new alkalinity in the combined water + sediment system, but rather represents a net transfer of sedimentary Fe–ANC to the water column, and a permanent depletion of the sedimentary Fe–ANC.

Inorganic sulfur may, however, be buried in forms other than FeS. For example, elemental sulfur must form, at least temporarily, in lake sediments:

(11) $$SO_4^{-2} + 2\,H^+ + 3\,H_2S \rightarrow 4\,S^\circ + 4\,H_2O$$

because pyrite, which forms by the reaction (Berner 1984):

(12) $$FeS + S^\circ \rightarrow FeS_2$$

has been identified in lake sediments (Cook 1981; Kelly and Rudd 1984; Davison et al. 1985; Nriagu and Soon 1985). The overall reaction for the formstion of pyrite from Fe(III) oxides and sulfate is then:

(13) $$15CH_2O + 8SO_4^{-2} + 4FeOOH + 16H^+ \rightarrow 4FeS_2 + 15CO_2 + 25H_2O$$

Note that 16 H^+ are consumed by this reaction, although the same number of moles of Fe(III) have been reduced as in Equations (2 and 10). Whereas consumption of the 8 H^+ in (10) was entirely associated with Fe(III) reduction and the conversion of Fe-ANC to FeS, an additional 8 H^+ are consumed in (13) by sulfate reduction and the formation and burial of 4 moles of pyrite which sulfur has an average oxidation state of −1 rather than −2 as in FeS. In terms of the species which have been removed from solution, and which would be measured in a water column ion budget, the ratio of $H^+:SO_4^{2-}$ consumed is still 1:1 as for earlier reactions. The difference here is that half of the alkalinity transferred to the water column represents the depletion of the sedimentary Fe-ANC whereas the other half represents the production of "new" alkalinity from the transformation of SO_4^{2-} to S(−1).

Organic Sulfur

Some recent papers have concluded that organic sulfur compounds, either carbon-bonded sulfur or ester sulfates, constitute a major part of the sulfur inventory in lake sediments (King and Klug 1982; Mitchell et al. 1984; Nriagu and Soon 1985). Sulfur can occur in a variety of oxidation states in organic compounds (Table 1). The stoichiometry of ester sulfate formation can be represented as:

(14A) $$ROH + H^+ + SO_4^{2-} \rightarrow RO{-}SO_3^- + H_2O$$

or

Table 1. Oxidation states of sulfur in compound classes which occur in natural aquatic systems.[a]

Oxidation state	General structure	Nomenclature
−2	$R-S-H$	Thiol, mercaptan Hydrogen sulfide (R = H)
−2	$R-S-R'$	Sulfide, thioether
−1	$R-S-S-R'$	Disulfide
0	$R-\overset{\overset{O}{\|}}{S}-R'$	Sulfoxide
+2	$R-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-R'$	Sulfone
+4	$R-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-OH$	Sulfonic acid
+6	$RO-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-O^-$ or $RO-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-OR'$	Ester sulfate
+6	$^-O-\underset{\underset{O}{\|}}{\overset{\overset{O}{\|}}{S}}-O^-$	Sulfate

[a]See review by Balzer (1981) for examples of organic compounds in each class. R and R′ may represent organic carbon moieties, H as noted, or nitrogen functional groups (not shown). Sulfur contained in inorganic forms: FeS, pyrite (FeS_2), elemental sulfur, sulfur dioxide (SO_2), and sulfurous acid H_2SO_3 has oxidation states of −2, −1, 0, +4, and +4, respectively.

$$ROH + R'OH + 2H^+ + SO_4^{2-} \longrightarrow RO{-}SO_2 - OR' + 2H_2O \tag{14B}$$

where R and R′ are organic carbon moities.

Carbon-bonded sulfur compounds can be produced from sulfate via assimilatory sulfate reduction. Formation of proteins is one example. The common amino acids, cysteine, methionine, and cystine, contain sulfur in the thiol, sulfide, and disulfide forms (Table 1), respectively. Carbon-bonded sulfur compounds may also form diagenetically by reaction of organic matter with H_2S (De Roo and Hodgson 1978), for example by replacement of a carbon-bonded oxygen atom by a sulfur atom from H_2S:

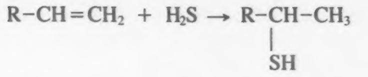
$$ROH + H_2S \longrightarrow RSH + H_2O \tag{15A}$$

or

$$ROR' + H_2S \longrightarrow RSR^1 + H_2O \tag{15B}$$

or by the addition reaction of H_2S with a C=C double bond:

(16)



$$R{-}CH{=}CH_2 + H_2S \rightarrow R{-}\underset{\underset{SH}{|}}{CH}{-}CH_3$$

where R and R′ are as defined above. One example of the type of diagenetic reaction which may occur in lake sediments, the reduction of carbohydrates by H_2S, is illustrated by the reaction between sucrose, dodecane, and H_2S studied by Mango (1983):

$$2C_{12}H_{22}O_{11} + 3C_{12}H_{26} + 7H_2S \longrightarrow 4C_6H_8S + 3C_{12}H_{20}S + 22H_2O \tag{17}$$

Note that all of the protons associated with H_2S have been converted to H_2O, unlike the precipitation of metal sulfides where the protons associated with the H_2S are released back into solution as free H^+ (eq. 8).

Sulfate and H^+ are consumed at a H^+:SO_4^{2-} ratio of 1:2 (eq. 14A) during the formation of ester sulfate anions ($ROSO_3^-$). During the formation of uncharged organic sulfur compounds, SO_4^{2-} and H^+ are consumed at a 1:1 equivalent ratio as can be seen in (14B) or by combining (6) with (15A, 15B, 16, or 17). If these products are formed in lake sediments using SO_4^{2-} that has diffused from the water column as the source of sulfur (Nriagu and Soon 1985; Rudd et al. 1986b), then the sulfate must be accompanied by an equivalent transfer of cations to the sediments to maintain charge balance. Formation of uncharged organic sulfur compounds consumes SO_4^{2-} and H^+ on a 1 : 1 basis, so it is reasonable that H^+ would be the co-diffusing cation and the ratio of (H^+ loss):(SO_4^{2-} loss): (SO_4^{2-} loss) from the water column would be 1:1. However, base cations may constitute a portion of the co-diffusing cations if the final product is a charged species such as $R{-}O{-}SO_3^-$, in which case the ratio of (H^+ loss) : (SO_4^{2-} loss) from the water column may be less than 1:1. As yet we know of no experimental data showing loss of SO_4^{2-} from lakewater balanced to a measurable extent by base cation loss rather than by H^+ loss.

Long-Term Capacity for Acid Neutralization

Cook (1981) measured the burial rate of total Fe in the hypolimnion sediments of one ELA lake to be 20 $mmol \cdot m^{-2} \cdot yr^{-1}$. Under steady state conditions this burial rate equals the rate of Fe supply to the lake and represents an upper limit for the rate of new Fe–ANC supply to the lake if all of the Fe is supplied as reactive FeOOH, i.e., neglecting Fe associated with aluminosilicates. This maximum rate of Fe-ANC supply is only ≃30% of the rate of atmospheric H^+ deposition in the northeastern United States (Driscoll and Likens 1982). Because the rate of H^+ loading, even neglecting H^+ supply from the watershed, can exeeed the maximum rate of Fe–ANC supply, the inventory of Fe–ANC in lake sediments that has accumulated over many years may eventually become exhausted if burial of iron sulfides is a major sink for SO_4^{2-} added to the lake as acid precipitation. The long-term capacity of lakes to neutralize acid precipitation will therefore depend, in part, on the inventory and rate of supply of Fe–ANC, as well as the relative amounts of FeS and FeS_2 buried because of the factor of two difference in the acid neutralized per mole of Fe–ANC consumed

Perhaps more important than Fe–ANC, the long-term capacity of lakes to neutralize acid will depend on the inventory and the rate of supply of reactive organic matter, which is necessary both to maintain anoxic conditions and to form organic sulfur compounds. If most of the sulfur in lake sediments occurs as organic sulfur compounds (King and Klug 1982; Mitchell et al. 1984; Nriagu and Soon 1985), and if this organic sulfur is formed using lakewater SO_4^{2-} as the sulfur source (Nriagu and Soon 1985; Rudd et al. 1986b), then the acid neutralization associated with SO_4^{2-} consumption in lake sediments may be dominated by the formation of organic sulfur compounds rather than by the burial of Fe sulfides.

Many problems and uncertainties still exist in the methods to identify sedimentary sulfur species (Nriagu and Soon 1985; Cornwell and Morse 1985; R. Howarth, pers. comm. 1986). One possible example of the misidentification of sedimentary sulfur species can be found in Nriagu and Soon (1985). Surface sediments of polluted lakes in the Sudbury, Ontario area were shown to have C/S ratios of $\simeq 1.5$ (0–4 cm, Kelley Lake) and 3.3–3.9 (0–8 cm McFarlane Lake), where 30–35% and 86–93% of the total sedimentary sulfur, respectively, were identified as being organic sulfur (ester sulfate plus carbon bonded sulfur). If these values are correct, then at a minimum about one carbon atom in 4 to 5 must be bonded to a sulfur atom, either directly or through oxygen in ester sulfates. The ratio would be even lower if a significant part of the sulfur occurs in sulfide (C–S–C) forms. Such a large fraction of C–S bonds seems unlikely for natural organic matter, and it seems more likely that some inorganic sulfides were misidentified as organic sulfur compounds.

Sedimentary sulfur species are generally assayed via sequential extraction schemes (e.g., Landers et al. 1983; Dickman and Thode 1985; Nriagu and Soon 1985), making it difficult to compare results obtained by laboratories using different techniques, especially since some sulfur classes are calculated by difference. Many laboratories are currently developing and testing techniques to measure sedimentary sulfur species. A thorough standardization and intercalibration of these techniques is necessary to provide reliable characterization and quantification of the chemical forms of sulfur buried in lake sediments.

Stoichiometric relationships between acid neutralization and SO_4^{2-} consumption can be written for the various possible fates of sulfur buried in lake sediments. Within-lake acid consumption can be an important defense against acidification by acid precipitation (Cook et al. 1986; Schiff and Anderson 1987a, b). However, the long term capacity of lakes to neutralize acid by internal biogeochemical processes depends on the chemical form of the final product of SO_4^{2-} consumption. Reliable determination of the chemical forms of sulfur in lake sediments and complete mass budgets for sulfur in lakes are required before quantitative predictions can be made about future trends in the acidification of lakes.

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Experimental Acidification of a *Sphagnum*-Dominated Peatland: First Year Results

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A poor fen (3.6 ha) in northwestern Ontario was experimentally acidified with HNO_3 and H_2SO_4 to study the effects of acid precipitation on the dominant *Sphagnum* species and on the chemistry of the surface waters and pore waters. The nitrate concentration in the surface waters of the fen increased from $< 0.07\ \mu eq \cdot L^{-1}\ NO_3^- - N$ to between 20–70 $\mu eq \cdot L^{-1}$ during or just after each acidification event. Subsurface $NO_3^- - N$ concentrations remained unchanged and surface $NO_3^- - N$ concentrations returned to background levels within a day. The mire as a whole retained over 99% of the added $NO_3^- - N$, probably due to nitrate assimilation by the *Sphagnum*. Sulfate concentrations in the surface pools increased from 5 $\mu eq \cdot L^{-1}$ to 130 $\mu eq \cdot L^{-1}$ with acidification and gradually decreased in the following 7 to 14 days. Pore water concentrations of $SO_4^=$ decreased and H_2S increased indicating sulfate reduction even in the highly acidic environment (pH 4). The decrease in concentration of $SO_4^=$ in the surface pools due to SO_4 reduction and assimilation was not as rapid as the decrease in NO_3^- concentration due to plant assimilation. The dominant *Sphagnum* species increased in growth with the added $NO_3^- - N$ and $SO_4^=$, particularly in the oligotrophic areas of the mire. In the minerotrophic area, only those species isolated from the water table showed increased growth due to acidification. Results here indicate that in the early stages of acidification the mire is capable of taking up the added NO_3^- and $SO_4^=$, probably in an assimilatory reduction process which generated additional alkalinity in the mire.

On a artificiellement acidifié un marécage improductif (3,6 ha) du nord-ouest de l'Ontario à l'aide de HNO_3 et de H_2SO_4 afin d'étudier l'incidence des préoccupations acides sur la principale espèce de *Sphagnum* et sur la chimie des eaux superficielles et interstitielles. Pendant ou juste après l'apport d'acides, la concentration de nitrate dans les eaux superficielles a augmenté de 0,07 $\mu eq \cdot L^{-1}\ NO_3^- - N$ jusqu'à un niveau situé entre 20 et 70 $\mu eg \cdot L^{-1}$. Les concentrations de $NO_3^- - N$ dans les eaux sous la surface n'ont pas changé tandis que les concentrations superficielles sont revenues à la concentration ambiante en moins d'une journée. Plus de 99 % du $NO_3^- - N$ étranger a été retenu dans le marécage, probablement à cause de l'assimilation du nitrate par *Sphagnum*. Les concentrations de sulfate dans les mares sont passées de 5 $\mu eq \cdot L^{-1}$ à 130 $\mu eq \cdot L^{-1}$ à la suite de l'acidification et ont graduellement diminué au cours des journées 7 à 14. Les concentrations de $SO_4^=$ dans les eaux interstitielles ont diminué tandis qe celle de H_2S ont augmenté, ce qui révèle une réduction du sulfate même en milieu fortement acide (pH 4). Dans les mares superficielles, la baisse de la concentration de $SO_4^=$ due à la réduction et à l'assimilation du $SO_4^=$ n'était pas aussi rapide que la baisse de la concentration de NO_3^- à la suite de l'assimilation végétale. Une croissance marquée de l'espèce dominante de *Sphagnum* a suivi l'apport de $NO_3^- - N$ et de $SO_4^=$, surtout dans les zones oligotrophes du marcécage. Dans la zone minérotrophe, seules les espèces isolées de la nappe aquifère ont montré une croissance accrue entraînée par l'acidification. Les résultats indiquent qu'au début de l'acidification, le marécage peut absorber le NO_3^- et le $SO_4^=$ ajoutés, probablement par réduction avec assimilation, ce qui engendre une alcalinité supplémentaire du marécage.

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Acid precipitation is widely known to cause acidification of lakes (Schindler et al. 1980) and of forest soils (Abrahamsen 1980; Wiklander 1980; Ulrich 1980). The effects of acid precipitation on wetlands are less well studied.

There is some evidence that northern wetland communities, i.e. bogs and poor fens, may be vulnerable to acid precipitation. Blanket bogs near industrial areas in the southern Pennines of Great Britain have lost most species of *Sphagnum*, the dominant peat former (Tallis 1964). Ferguson et al. (1978) showed that the growth of several *Sphagnum* species was inhibited by HSO_3^-, $SO_4^=$, and SO_2. The sensitive species in their study were the same ones that disappeared from the bogs studied by Tallis (1964). The elements, nitrogen and sulfur have been implicated in the disappearance of *Sphagnum* from British bogs (Ferguson and Lee 1983a, 1983b; Lee et al. 1987). Belgian moorland pools have become more acid and Vangenechten and Vanderborght (1980) hypothesize that industrial acidification has decreased the pH and increased the $SO_4^=$ concentration in these bog lakes. A recent review by Gorham et al. (1984) summarizes the known detrimental effects.

The purpose of this study was to determine the effects of known concentrations and volumes of sulfate, nitrate, and hydrogen ion on the vegetation and chemistry of a poor fen in northwestern Ontario, an area which has had generally low levels of atmospheric pollutants. The major hypotheses which we investigated include: (1) the pH of the fen waters will be reduced by the addition of H_2SO_4 and HNO_3; (2) sulfate and nitrate reduction and absorption will be increased with acidification; (3) growth of the primary vegetation (*Sphagnum* sp.) will be increased. To understand the major differences in the two primary regions of the fen, the oligotrophic central area and the minerotrophic edge area (lagg), we present background chemical results collected weekly during 1984. These data were collected before the acidification events that also occurred in 1983 and 1984. Then we present results of the 1 ½ yr of acidification experiments on the chemistry and vegetation of the mire. Each experiment is an acidification of a 3.51 ha area, with chemical samples of water collected before, during and just after the 5 h acidification event. In addition, several field and laboratory experiments are presented to show absorption of NO_3 and SO_4 by *Sphagnum* vegetation.

Site Description, Experimental Design, and Methods

Study Area

The research site (Fig. 1) is a 3.67 ha poor fen (Mire 239) located in the Experimental Lakes Area (ELA) of northwestern Ontario (Lat. 49°40′N, Long. 93°43′W). The 239 Mire is part of a 10.28 ha terrestrial subbasin of Lake 239. The upland part of the subbasin and 41% of the mire burned in 1974. The subbasin (excluding the mire) burned again in 1980. Prior to burning, the dominant overstory vegetation in the upland area was mature jackpine (*Pinus banksiana*), and black spruce (*Picea mariana*). The upland part of the subbasin is currently sparsely covered with young jack pine, trembling aspen, (*Populus tremuloides*) balsam poplar (*Populus balsamifera*), pin cherry (*Prunus pennsylvanica*), saskatoon (*Amelanchier alnifolia*), and alder (*Alnus crispa*).

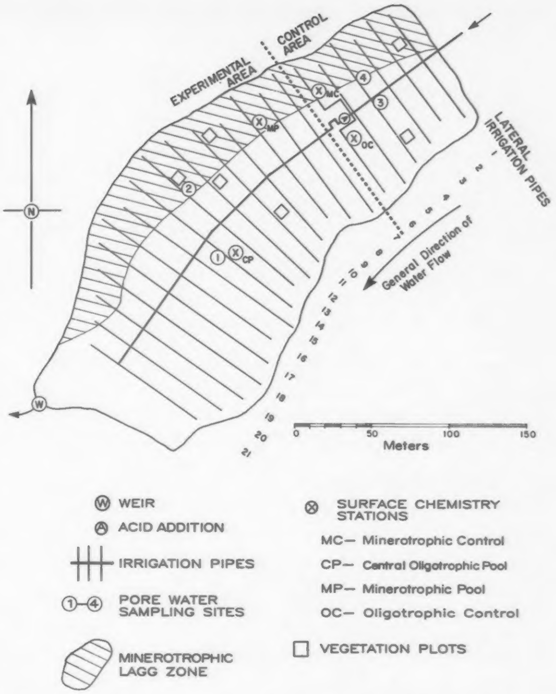

FIG. 1. Mire 239 with the irrigation system for acidification of 3.51 ha shown.

The vegetation of mire 239 has been described in detail by Vitt and Bayley (1984) and is dominated by a tree layer, when present, of black spruce, a shrub layer of *Chamaedaphne calyculata* and *Ledum groenlandicum*, a herb layer of *Smilacina trifolia*, and *Carex trisperma*, and a ground layer of *Sphagnum angustifolium* forming carpets and lawns and *S. magellanicum* on lawns and hummock-sides.

The mire can be divided into two major physiographic areas, recognizable by differences in vegetation. The central oligotrophic portion of the mire is characterized by an abundance of *Andromeda glaucophylla*, *Kalmia polifolia*, and *Vaccinium vitis-idaea*. Distinct minerotrophic indicators are lacking. The southern part of this area was burned in 1974 and except for several seral plant species (*Lycopodium annotinum*, *Gaultheria hispidula*, and juvenile individuals of *Pinus banksiana*) is similar to the northern unburned portion. The vegetation suggests that the central, burned and unburned, portions of the mire are nutrient-poor and not much influenced by minerotrophic water.

The second major vegetation type occurs along the northern and western edges of the mire, and contains indicators of minerotrophy that are absent in the central mire portions. Included in these species are *Equisetum sylvaticum*, *Alnus rugosa*, *Salix planifolia* and *Calamarostis canadensis*. These species indicate a lagg fen zone with a greater degree of soil water influence than present in the central portions of the mire.

Based on the species above, two mire types are recognized in this study, (1) minerotrophic areas (defined by the presence of *Equisetum sylvaticum*, *Alnus rugosa*, and *Salix planifolia*) and (2) oligotrophic areas (defined by the lack of these species). This oligotrophic area is not considered to be ombrotrophic and hence this mire can be classified as a poor fen (Sjors 1950).

Acidification Experiments

The purpose of the experiment was to lower the pH of precipitation on the mire equivalent to the pH of most acid precipitation in the eastern United States and Canada (≈pH 4). Since the long-term average natural pH of precipitation at ELA is ≈4.9, the acid added must reduce the pH by 1 unit. Based on long-term precipitation records, this required 1804 equivalents of strong acid annually. Since the experiment began mid season in 1983, half this amount of acid was added (908 equivalents) in that year.

There is some evidence (NAS 1981) that the sulfur deposition rate has leveled or even declined in eastern North America. In contrast, the nitric acid deposition rate is still increasing and is expected to account for almost half of the total acid deposition by the year 2000 (OTA 1984; NAS 1981). Based on this projection, 50% H_2SO_4 and 50% HNO_3 were applied as acids on an equivalent basis. The acid was actually sprayed on the bog at pH 3 to reduce the volume of lake water applied to the bog and to keep the experimental disturbance of the hydrologic and cation budgets as low as possible.

The mire was irrigated with acid using a pipe distribution network to deliver water to 160 sprinkler heads, an irrigation pump to supply water, a hydrant located near the pump on the discharge side to regulate delivery of water to the mire, an in line flow meter to monitor pumping rates, and pressure gauges located at the pump and on the distribution lines. Details of the irrigation system are found in Beaty (1987).

The irrigation pump was located 150 m from the northeast end of the mire on the shore of nearby Roddy Lake. Lake water from a depth of about 1 m was pumped up 6.7 m to the back of the subbasin and down 3.6 m to the mire surface (net elevation difference 3.1 m).

The area of the mire receiving acid plus Roddy Lake water (experimental portion) was 2.66 ha. In addition, a 0.85 ha control portion received only Roddy Lake water. A small portion of the mire near the outflow (0.16 ha) was not irrigated.

The water in the control area flowed in a downward gradient into the experimental area, particularly on the edge of the mire. Flow into the central oligotrophic area was more limited. Runoff from the surrounding watershed also flowed primarily into the edge of the mire rather than through the central portion of the mire. The outflow of the fen is bedrock controlled and therefore groundwater seepage is minimal. The mire is located 1.2 km west of the main ELA meteorological station at approximately the same elevation.

In 1983, 3 monthly acidification events were applied, 3 Aug., 31 Aug., and 11 Oct. In 1984, monthly applications of acid started in May. Each contained H^+ equivalent to 16% of average annual deposition of precipitation at pH 4. In each experiment, the bog was initially sprayed with lake water for 10–20 min to wet the surface. Acid was added to the water in the experimental part of the bog, resulting in spray with a pH of 3.0. Irrigation lasted 4–5 h, followed by 20–30 min of rinse at a higher pH (5–7) to mimic a natural acid rainstorm and avoid direct damage to the vegetation. The amount of water and equivalents of acid added in each acidification event are presented in Table 1.

TABLE 1. Additions to Mire 239 in 1983 and 1984 during the acidification experiments.

Date	Water pumped m^3	Acid pumped (Equivalents) H_2SO_4	HNO_3	Total acid
1983				
Aug. 3	470	163	142	305
Aug. 31	343	165	143	308
Oct. 11	243	158	137	295
	1 056	486	422	908
1984				
May 23	301	167	145	312
June 20	344	170	148	318
July 18	468	168	147	315
	1 113	505	440	945

Experimental Design and Chemical Methods

The experimental design for the field sampling consists of four treatment areas: oligotrophic experimental, minerotrophic experimental, oligotrophic control, and minerotrophic control (Fig. 1). In 1983 surface water samples were collected only in the oligotrophic central pool and in a small tree hollow near the edge of the mire characteristic of more mineral conditions (called the minerotrophic pool) (Fig. 1). Both stations received acidified water during the experiments. In 1984, two additional surface water stations were added in the control area of the mire (oligotrophic control and minerotrophic control).

Water samples from surface pools were collected weekly during the ice-free season as well as before, during, and after each acidification event. Samples were also collected 12–24 h after each acidification event.

Pore waters were measured monthly at four sampling sites within the mire. Pore water samples were also collected before, immediately after acidification and 12–18 h after each experiment. Sites 1 and 2 were located in the experimental area of the mire, sites 3 and 4 were in the control region of the mire (Fig. 1). Sites 1 and 3 were characteristic of the nutrient poor, oligotrophic region of the mire and sites 2 and 4 were characteristic of the more nutrient rich, minerotrophic lagg region of the mire. All of these sites were located in hollows. Pore water samples were obtained at 5 cm intervals from the top of the water table to a depth of approximately 35 cm. Details of the hollow stainless steel sampling tubes are found in Behr (1985).

All surface water samples were analyzed for NO_3^-–N, NH_4^+–N, $SO_4^=$, pH, and alkalinity. Nitrate and ammonium nitrogen were analyzed within 4 h using a Technicon autoanalyser as described in Stainton et al. (1977). The waters were frequently highly colored, so analyses were corrected for natural color, which was subtracted from the NO_3^-–N and NH_4^+–N values. Sulfate was measured using an ion chromatograph (Dionex).

Surface water pH was measured within 4 h of sampling using an Orion pH meter and combination electrode calibrated against dilute acid standards. Gran alkalinity was measured using an automated titro processor (Linsey and Braund 1984). The negative alkalinities found in some of the mire waters were determined by addition of known quantities of HCO_3^- to the samples and titrated as above.

Pore water samples were analyzed for $SO_4^=$, H_2S, NO_3^-, NH_4^+, and H^+ ion concentration using the methods described

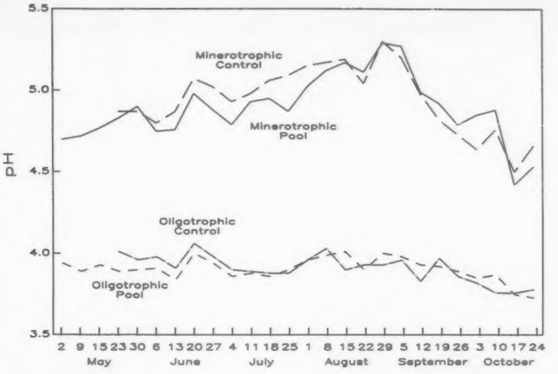

FIG. 2. Weekly pH of experimental surface pools (minerotrophic pool and oligotrophic pool) and control surface pools (minerotrophic control and oligotrophic control) in 1984 before acidification events.

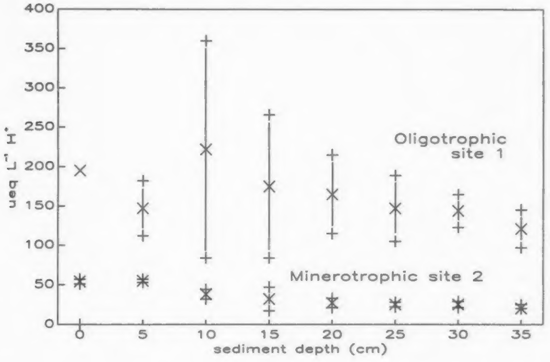

FIG. 3. Hydrogen ion concentration of pore waters under natural conditions in the minerotrophic and oligotrophic areas in 1984.

above. Hydrogen sulfide was analyzed by the method of Stainton et al. (1977). Reagents were injected through a 3-way valve to prevent contamination by atmospheric oxygen. The H_2S analyses were normally completed within 3 h of sample collection. Sulfate samples were filtered with a Swinnex® filtration device equipped with a 0.22 µm filter (Nuclepore) that had been rinsed with distilled deionized water. Samples were refrigerated until analyzed with the ion chromatograph.

Growth Measurements of *Sphagnum*

Four experimental and control sites for *Sphagnum* studies were established on Mire 239 in August, 1983. The four sites correspond to the four treatment areas of the mire: oligotrophic control, minerotrophic control, oligotrophic experimental, and minerotrophic experimental.

Each plot contained 120 stainless steel cranked wires (Clymo 1970) positioned along four hummock top-mid hummock-lawn transects, each of approximately 30 stakes. *Sphagnum* growth was measured relative to the wires, with data organized as *Sphagnum angustifolium* = lawns, *S. magellanicum* (or any combination with *S. angustifolium* and *S. fuscum* = mid hummocks), and *S. fuscum* = hummock tops.

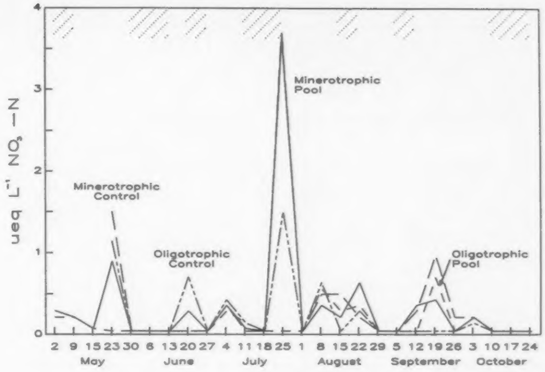

FIG. 4. Weekly concentration of $NO_3{-}N$ in mire surface pools in 1984 increased after rainfall, but were unaffected by acidification events.

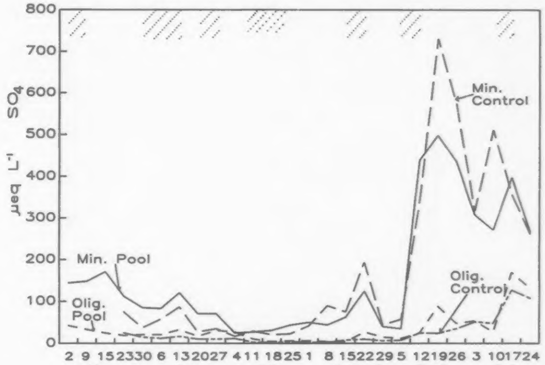

FIG. 5. Weekly concentration of sulfate in mire surface pools in 1984.

Two time periods were examined, the first between 22 May and 18 June, 1984, and the second between 19 June and 10 July, 1984.

Nitrate Uptake by *Sphagnum*

To determine whether NO_3^- was taken up directly by *Sphagnum*, polyethylene funnels were implanted in *Sphagnum* hummocks in the experimental and control sites. A portion of *Sphagnum* was removed from a hummock, a funnel placed in the depression, and the moss carefully replaced. Tygon tubing was attached to the lower end of the funnel in the peat; a prewashed scintillation vial was attached to the other end of the tubing to retain water which passed through the *Sphagnum*. Eleven funnels were implanted for each of *S. magellanicum* and *S. fuscum*, three in the oligotrophic control areas, and four in each of the minerotrophic and oligotrophic experimental areas. Spray percolate collected in the vials was analyzed for nitrate immediately after the irrigation experiment.

Analysis of $NO_3^-{-}N$ uptake by *Sphagnum angustifolium* differed from the other *Sphagnum* species due to its lawn habitat. Three replicate samples of pool water were collected from around *S. angustifolium* plants in each of the three sites (oligotrophic control, oligotrophic, and minerotrophic

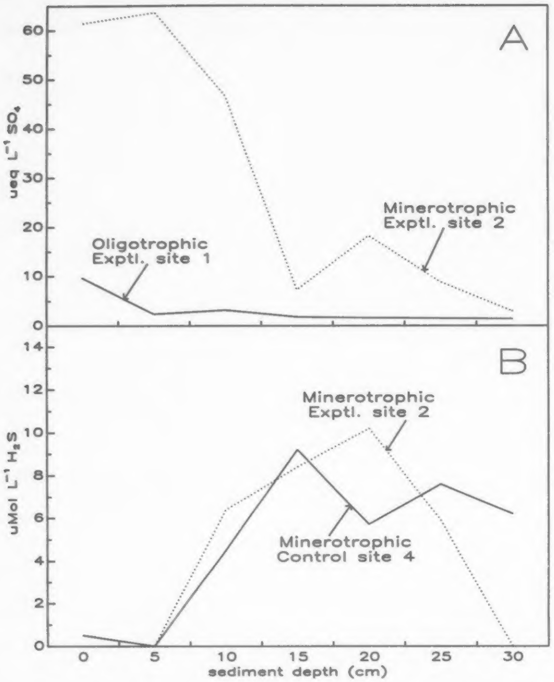

FIG. 6. (A) Sulfate concentration in pore waters prior to acidification in the central oligotrophic area (Site 1) and the minerotrophic area (Site 2) on 23 May 1984; (B) Hydrogen sulfide concentrations in pore waters prior to acidification in the experimental and control minerotrophic sites on 20 June 1984.

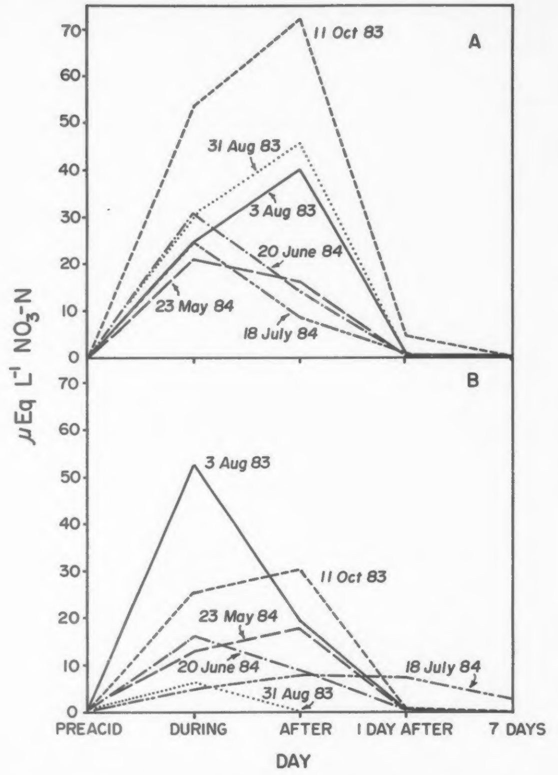

FIG. 7. (A) Concentration of NO_3−N in the oligotrophic central pool before and after acidification experiments. Samples collected prior to acidification, during acidification (2 h after start), < 1 h after termination, 1 d after and 7 d after acidification; (B) Concentration of NO_3−N in the minerotrophic pool before, during and after acidification experiments.

experimental) before and after spraying. Additionally, *S. angustifolium* plants from the same pools were squeezed for analysis of nitrate, before and after spraying.

A laboratory experiment of the loss of nitrate in the acid spray due to uptake by *Sphagnum* was also made. This experiment proceeded as follows: *Sphagnum fuscum*, *S. magellanicum*, and *S. angustifolium* plants were collected and cut into segments. Next, pre-washed, 100 mL jars were filled with duplicate samples of (1) whole plants, (2) capitula, (3) two cm of green stems and (4) two cm of brown stems. Acid spray medium was added to fill the jars at an initial NO_3^-−N concentration of 668 $\mu eq \cdot L^{-1}$ NO_3^-−N. Each jar was sampled for nitrate concentration at intervals of 1, 12, and 20 h after initiation of the experiment. Nitrate concentration was analyzed less than 12 h after collection, with results based on dry weight of *Sphagnum* plants and expressed as % loss of nitrate. Capitula and green stem data are not included in this paper because results are similar to those obtained from the whole plant.

Results

Background Chemistry of Mire Water Before Acidification Events

Mire 239 has two distinct regions based on chemical characteristics that parallel the vegetation regions. The pH of the center pool, which characterizes the oligotrophic region, averaged 4.03 in 1983 and 3.90 in 1984 (93 and 126 $\mu eq \cdot L^{-1}$, respectively) (Fig. 2). The pH of the minerotrophic pool averaged 4.84 in 1984 (14 $\mu eq \cdot L^{-1}$) (Fig. 2). The pH of the minerotrophic surface waters was more variable in both time and space than was the central oligotrophic region. The lowest pH values in the mineral pool were detected in the spring.

There was also a considerable difference in the pH of pore waters of the oligotrophic and minerotrophic sites. The oligotrophic sites had significantly lower pH than the minerotrophic lagg sites. The pH was usually in the range of 3.7 to 4.1 (90−200 $\mu eq\ H^+ \cdot L^{-1}$) in the oligotrophic sites and was rather constant with depth (Fig. 3) except during the dry autumn. The pH in the minerotrophic sites was approximately 4.2 to 4.5 (30−60 $\mu eq \cdot L^{-1}$) at the surface of the water table, increasing to 4.5 to 5.0 (10−30 $\mu eq \cdot L^{-1}$) by 40 cm.

Nitrate nitrogen concentrations in all surface pools of the mire were very low (Fig. 4), usually <0.5 $\mu eq \cdot L^{-1}$ or undetectable except after natural precipitation events. The minerotrophic lagg generally had slightly higher concentrations than did the oligotrophic area, although the differences were not statistically significant. The lowest concentrations were detected in the central oligotrophic pool (mean concentration 01.4 $\mu eq \cdot L^{-1}$). No nitrate was detected in the pore waters of the mire except during acidification experiments (see later).

Ammonia-N concentrations in the mire were 4–18 times higher than NO_3^-–N (data not shown) even though NH_4^+–N and NO_3^-–N in natural precipitation were approximately equal (1970–83 mean: 284 μeq·L^{-1} NH_4^+–N, 253 μeq·L^{-1} NO_3^-–N) (Linsey et al. 1987). Ammonia-N concentration showed a sharp peak in 1984 on July 19, 3–4 d after 28 mm of rain. Nitrate-N concentration showed a similar peak 1 wk later (Fig. 4) after 5.2 mm of additional rainfall.

Pore water concentrations of NH_4^+–N generally increased with depth in the peat (data not shown). Concentrations of NH_4^+–N in three of the four pore water sites generally remained below 100 μeq·L^{-1}. Only the oligotrophic control area (site 4) near the head of the mire showed the high concentrations of NH_4^+–N (300–3000 μeq·L^{-1}) with depth reported by other authors (Hemond 1983; Urban 1983; Waughman 1980).

Sulfate concentrations within the mire varied significantly with respect to time and space. The surface waters of the minerotrophic region (lagg) had higher $SO_4^=$ concentrations than the central oligotrophic region of the mire (Fig. 5). This difference was evident throughout the ice-free season but was most pronounced shortly after the spring snowmelt, demonstrating the greater influence of upland runoff on the lagg areas. The difference was even greater in the fall when dramatic increases in $SO_4^=$ concentrations in the mineral pools were detected after a period of prolonged drought and low water table followed by several days of heavy rainfall.

A typical preacidification $SO_4^=$ pore water profile from each of the regions in the mire is shown in Fig. 6A. Generally, highest concentrations of $SO_4^=$ at the minerotrophic sites were at or near the surface, decreasing with depth, probably due to sulfate reduction. Surface concentrations of $SO_4^=$ were as high as 100–200 μeq $SO_4^=$·L^{-1} shortly after spring snowmelt or after a significant amount of precipitation. Later in the season or after extended dry periods, $SO_4^=$ concentrations fell below 10 μeq·L^{-1}.

Increased concentrations of H_2S with depth were observed only in the minerotrophic lagg sites under natural conditions. Sulfide concentrations in these sites ranged from undetectable to 20–30 μeq S·L^{-1} (Fig. 6B). In general, the highest concentrations occurred after significant $SO_4^=$ inputs to the system (Behr 1985). In 1 1/2 yr of sampling, measurable amounts of H_2S were found in the two central oligotrophic sites only once or twice (i.e. >2 μeq S·L^{-1}).

Effects of Acidification on Mire Water Chemistry

The Fate of Nitrate

Nitrate in the surface water of both the central oligotrophic pool and the mineral pool increased to 7–71 μeq NO_3^-–N·L^{-1} during and just after irrigation (Fig. 7A, B). Twelve to 24 h after irrigation, the NO_3^-–N concentration in both areas returned to background concentrations (usually <1 μeq·L^{-1}). With spray irrigation concentrations averaging 535 μeq·L^{-1} and a dilution of 86% in the mire water (based on the measured dilution of silica), the expected concentration would have been 57–86 μeq·L^{-1}. The loss of the added NO_3^-–N could be due to denitrification, assimilatory NO_3^- reduction or dissimilatory NO_3^- reduction. High ammonium concentrations would indicate dissimilatory NO_3^- reduction, but little increase in ammonia was observed (data not shown).

The pore waters in the top 5 cm of peat had undetectable levels of NO_3^-–N immediately after acidification for four of the six acidification experiments. The other two experiments resulted in an increase in pore water NO_3^-–N concentration in the upper 10 cm, but these values generally returned to undetectable levels 1 d later. This loss of NO_3^- from pore waters may be due to denitrification by nitrate-reducing bacteria or by NO_3^- absorption by peat.

Even though the experimentally added nitric acid increased the NO_3^- input to the mire by 50% in 1983 (Table 2), the outflow of nitrate was negligible (0.4% of inflow). Nitrate is in short supply in bogs and poor fens and the annual mass balance calculations for the mire for the years 1971 through 1983 indicate that very little was exported (Bayley, unpubl. data).

The Fate of Sulfate

Sulfate concentrations in surface pools increased rapidly after addition of the sulfuric acid (Fig. 8A, B). For example, during July 1984 the oligotrophic pool increased from preacidification concentrations of 2.0 to 67 μeq $SO_4^=$·L^{-1} and the mineral pool increased from 31 to 50 μeq $SO_4^=$·L^{-1} during the experiment and increased to 88 μeq $SO_4^=$·L^{-1} just after the acidification. One day after irrigation the oligotrophic and minerotrophic pools had decreased to 31% and 44% of their respective maximum concentrations. Overall, sulfate concentrations decreased rapidly in the minerotrophic and oligotrophic pools after irrigation events (Fig. 8A, B). However the decrease in sulfate concentration was not as rapid as the decrease in NO_3^-–N concentrations.

In the control region of the bog, surface $SO_4^=$ concentrations increased after application of Roddy Lake water (Fig. 8C), but the increase in concentration in the control areas was much smaller than that in the experimental pools. The minerotrophic control pool increased from 21 to 32 μeq $SO_4^=$·L^{-1}, and the oligotrophic control pool increased from 2 to 10 μeq $SO_4^=$·L^{-1} directly after addition of Roddy Lake water.

Pore water $SO_4^=$ concentrations in the minerotrophic site increased after all six of the acidifications while only two of the six acidifications resulted in increased pore water $SO_4^=$ concentration in the oligotrophic site. Behr (1985) reports details of the pore water responses to acidification.

The occurrence of H_2S in pore water was restricted to the minerotrophic lagg sites. In all of the acidifications to date, the concentration of H_2S in the pore water of the minerotrophic experimental site (site 2) increased after acid application (Behr 1985).

The Effect on Hydrogen Ion

The pH in the oligotrophic and minerotrophic pools decreased slightly with addition of the acid but increased to preirrigation levels within 1 d (data not shown). The greatest decrease in pH detected in the oligotrophic pool was on 31 August 1983 when the pH went from 4.08 (83 μeq·L^{-1}) to 3.72 (191 μeq·L^{-1}) with the addition of acid. In the mineral pool the pH decrease was usually much smaller, typically 4.95 (11 μeq·L^{-1}) to 4.71 (19 μeq·L^{-1}). Both pools were obviously well buffered.

Contrary to the surface pools, the effect of acidification on the concentration of H^+ (ie. pH) in the pore water of the minerotrophic and oligotrophic sites was not consistent (data

TABLE 2. NO_3–N and SO_4 budgets for 239 Mire in 1983. Acid was added intermittently to the Mire during the latter half (Aug.–Oct.) of the 1983 ice free season.

	SO_4		NO_3–N	
	$g \cdot mire\ yr^{-1}$	$g \cdot m^{-2} \cdot yr^{-1}$	$g \cdot mire\ yr^{-1}$	$g \cdot m^{-2} \cdot yr^{-1}$
Natural precipitation	43 857	1.20	11 271	0.31
Acid precipitation (incl. Roddy Lake water)	34 351	0.94	5 936	0.16
Runoff into bog	44 313	1.21	23	0.001
Total input	122 521	3.35	17 230	0.471
Outflow	32 660	0.89	63	0.002
Assimilation or reduction	89 861	2.46	17 167	0.469
% retention	73		>99	

not shown). Some acidification events resulted in increases in H^+ concentration in the pore water, while others resulted in decreases in H^+ concentration immediately following the acid application.

Effects of Acidification on *Sphagnum* Growth

In situ *Sphagnum* growth increased with the addition of H_2SO_4 and HNO_3 to the mire. The most dramatic increase in stem length was observed in a lawn species, *Sphagnum angustifolium* (Fig. 9A, C, D). In general, the irrigation of the mire with acid significantly increased growth of lawn, mid-hummock and hummock species of *Sphagnum* in the oligotrophic part of the mire, whereas in the minerotrophic (lagg) portions, growth increased significantly only on the hummock-tops, which are isolated from ground water influences. Detailed comparisons of growth under experimental and control conditions in the oligotrophic and minerotrophic portions of the mire follow.

Comparison of Minerotrophic and Oligotrophic Experimental Conditions

Comparisons of growth between the minerotrophic and oligotrophic experimental sites for each of the three species showed no significant differences for *S. angustifolium*, *S. magellanicum*, and *S. fuscum* (Table 3). Under the experimental conditions, growth of *S. angustifolium* (lawns) was significantly greater than that of *S. magellanicum* (all $P < 0.001$) (mid-hummocks) and *S. fuscum* (hummock tops) (all $P < 0.001$, Fig. 9A). This was true for both time periods (22 May–18 June 1984 and 19 June–10 July 1984) (Table 3).

Thus, growth under experimental conditions in the minerotrophic and oligotrophic sites was similar for all three species. However, under both minerotrophic and oligotrophic experimental conditions, the lawn species *S. angustifolium* grew considerably more than either *S. magellanicum* or *S. fuscum* (Fig. 9A and Table 3).

Comparison of Minerotrophic and Oligotrophic Control Conditions

When growth under control conditions was compared between minerotrophic and oligotrophic sites for each of the three species, no significant differences were found for the growth of *S. angustifolium* (on lawns) or *S. magellanicum* (mid-hummocks) between the control minerotrophic and oligotrophic sites (Table 3). However, significant differences for both time periods ($P < 0.05$, and < 0.001) were found in *S. fuscum* (hummock tops) with higher growth values in the minerotrophic control area versus the lower, oligotrophic control.

Thus, growth of lawns and mid-hummock species under control conditions in the minerotrophic and oligotrophic sites was comparable for each time period within each species, while the hummock-top species grew more in the minerotrophic site. Growth of the lawn species (*S. angustifolium*) and mid-hummock species (*S. magellanicum*) was comparable; however, some differences were found between the growth of *S. angustifolium* and the hummock-top species — *S. fuscum*.

Comparison of Growth under Minerotrophic Conditions

Growth for *S. fuscum* (hummock-tops) was significantly greater in the experimental site ($P < 0.02$) for time period one (22 May–18 June 1984) (Fig. 9B) and significantly greater in the control site ($P < 0.05$) for time period two (19 June–10 July 1984). No significant differences were found between control and experimental sites in growth of *S. angustifolium* and *S. magellanicum* under minerotrophic conditions for either time period. Therefore we concluded that the addition of acid did not cause an increase in growth in lawns and mid-hummock habitats in the minerotrophic sites, perhaps because the lawns and mid-hummocks were already influenced by runoff water. The hummock-top species (*S. fuscum*) is microtopographically isolated from ground water influences, and would seem more likely to show effects due to acidification; this in fact occurred during the early spring growth period (Table 3).

Comparison of Growth under Oligotrophic Conditions

Growth of *S. angustifolium* (lawns) was significantly greater under experimental conditions than under control conditions for both 22 May–18 June ($P < 0.01$) and 19 June–10 July ($P < 0.001$, Fig. 9C). *Sphagnum magellanicum* growth (mid hummocks) was greater from 22 May–18 June ($P < 0.05$) under experimental conditions, but not so from 19 June–10 July. Growth of *Sphagnum fuscum* was greater under experimental conditions for both time period one (22 May–18 June) ($P < 0.001$, Fig. 9D) and time period two (19 June–10 July) ($P < 0.01$). Under these conditions mean values for growth of *S. angustifolium* were 12.4 and 7.5 mm for control conditions for time periods one and two, respectively; while under experimental conditions they were 22.6 and 17.4 mm. See Table 3 for standard deviations. Likewise for time periods one and

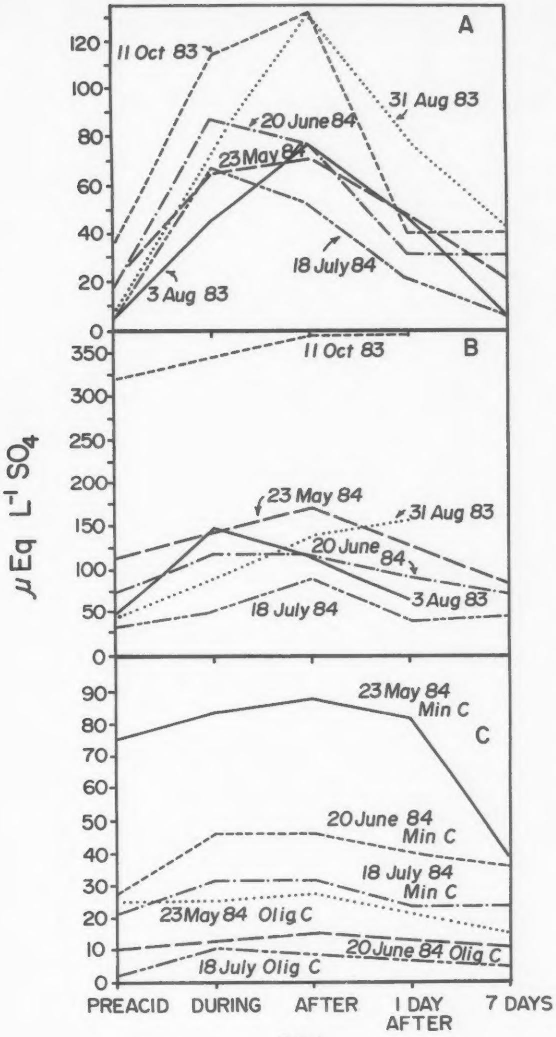

FIG. 8. (A) Concentration of sulfate in the experimental oligotrophic pool before and after acidification; (B) Concentration of sulfate in the experimental minerotrophic pool before and after acidification; (C) Concentration of sulfate in the control pools before and after water irrigation.

two, respectively, growth of *S. magellanicum* was 7.8 and 6.9 mm under control conditions and 11.3 and 9.2 under experimental conditions. Mean growth values of *S. fuscum* under control conditions were 3.3 and 3.5 mm, while under experimental conditions, they were 9.5 and 6.4 mm (Table 3).

Uptake of Nitrate by *Sphagnum*

Field Experiments

The hummock top species *S. fuscum* and mid-hummock species *S. magellanicum* removed over 97% of the nitrate in the acid spray (Table 4) during the 4 h of the acidification event. The nitrate was removed during the time the water flowed from the top of the *Sphagnum* mat to a funnel beneath the mat.

Analysis of pool water samples surrounding the lawn species *S. angustifolium* indicated low concentrations of nitrate (less than 1 $\mu eq \cdot L^{-1}$) in water surrounding the plant (Table 5). Nitrate released by plants when squeezed averaged about 53 $\mu eq \cdot L^{-1}$ from the oligotrophic control area. The concentration of nitrate in the pool water of the control area did not change after spraying with lake water (at <1 $\mu eq \cdot L^{-1}$), yet the concentration of nitrate in the water squeezed from the plant increased 240% (Table 5). Within the experimental areas, the pool water NO_3^-−N increased over 1000% after spraying, while the plant−water nitrate increased 2150 times in the minerotrophic area, and 5700 times in the oligotrophic area (Table 5).

Laboratory Experiments

Laboratory experiments with nitrate uptake confirm the findings of the field experiments. Two species of *Sphagnum* removed 100% of the nitrate from the water within 12 h (Fig. 10A). The third species, *S. magellanicum* removed over 93% in the same period. After 20 h, the nitrate concentrations went from 66.8 $\mu eq \cdot L^{-1}$ to <1 $\mu eq \cdot L^{-1}$ with all three species. The uptake of NO_3^-−N within the first hour was very rapid, ranging from 28 to 37 $\mu eq \cdot L^{-1} \cdot h^{-1} \cdot g^{-1}$ of *Sphagnum*. The capitula or growing tip of the *Sphagnum* took up nitrate (49 $\mu eq \cdot L^{-1} \cdot h^{-1} \cdot g^{-1}$) even more rapidly than did the whole plant or any of its other parts. Contrary to expectation, the brown dying segments of the *Sphagnum* species also took up nitrate rapidly (Fig. 10B). Within the first hour, the brown stems took up 13−28% of the available nitrate and by 12 h the brown stems had taken up from 79 to 100% of the nitrate. Since nitrate uptake was not dependent on actively growing plant material, ion exchange may be an important mechanism controlling this uptake.

Discussion and Summary

After a year of experimental application of H_2SO_4 and HNO_3, the three dominant *Sphagnum* species showed increased growth in response to the added acids. The decrease in pH in the minerotrophic and oligotrophic experimental pools in response to acidification was a temporary phenomenon, pH generally returned to pre-acidification values within 1 d. The increase in concentration of $SO_4^=$ and NO_3^-−N in surface pools was also only a temporary phenomenon (Fig. 7 and 8) and there was no increased outflow of $SO_4^=$ and NO_3^- from the mire due to acidification (Table 2).

British bogs have lost most of their dominant *Sphagnum* species over the last 200 yr in areas that receive high atmospheric deposition (Tallis 1964, 1973; Ferguson and Lee 1980). While we do not know why our results differ from those reported from Britain, there are obvious differences in the two situations. The British sites studies by Lee and coworkers have received high atmospheric deposition for hundreds of years, and even their relatively clean sites have higher deposition than the ELA site (Press and Lee 1982).

We do not expect our results to mimic the British situation since our poor fen has received increased acid for only 1 1/2 yr and may be highly buffered due to the high concentrations

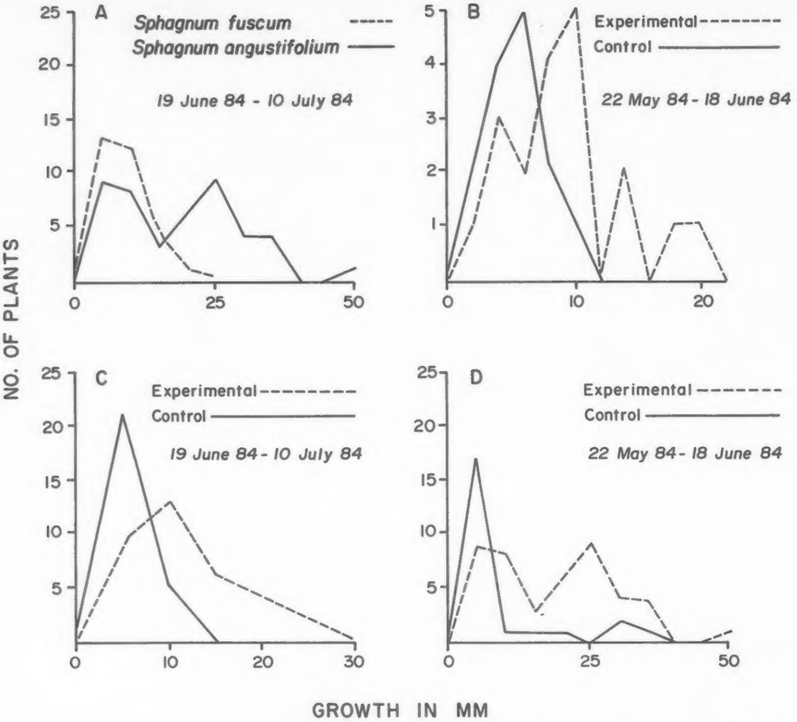

FIG. 9. (A) Comparison of growth (in mire) of *Sphagnum angustifolium* (on lawns) and *S. fuscum* (on hummock top) under oligotrophic experimental conditions (time period two); (B) Comparison of growth of *Sphagnum fuscum* under minerotrophic control and experimental conditions (time period one); (C) Comparison of growth of *Sphagnum angustifolium* under oligotrophic control and experimental conditions (time period two); (D) Comparison of growth of *Sphagnum fuscum* under oligotropohic control and experimental conditions (time period one).

TABLE 3. Mean growth (in mm ± standard deviation) for the three *Sphagnum* species in each of the plots. TP1 = Time Period one — May 22–June 18, 1984, TP2 = Time Period Two — June 19–July 10, 1984. Significant differences (*t*-test, two-tailed) between experimental and control pairs noted by lines, with level of significance between these discussed in the text and given under the line. *n* = number.

	Time Period	Minerotrophic control		Minerotrophic experimental	Oligotrophic control		Oligotrophic experimental
Sphagnum angustifolium (lawn)	TP1	14.2(9.8)		19.6(13.2)	12.4(12.)	0.01	22.6(13.0)
		n = 20		27	24		44
	TP2	12.8(11.9)		20.4(12.8)	7.5(9.8)	0.001	17.4(10.9)
		n = 16		26	23		44
Sphagnum magellanicum (mid hummock)	TP1	10.7(5.8)		10.1(6.6)	7.8(7.3)	0.05	11.3(7.8)
		n = 32		60	36		36
	TP2	9.1(4.8)		7.5(4.5)	6.9(6.6)		9.2(6.8)
		n = 29		50	36		34
Sphagnum fuscum (hummock top)	TP1	5.1(2.4)	0.02	8.7(4.7)	3.3(2.5)	0.001	9.5(5.9)
		n = 14		19	26		34
	TP2	8.2(3.8)	0.05	5.3(3.4)	3.5(2.4)	0.01	6.4(4.1)
		n = 13		22	32		30

TABLE 4. Nitrate–nitrogen uptake by *Sphagnum fuscum* and *S. magellanicum* in the mire. Data from analysis of water passed through implanted funnels (see Methods for details) and expressed as % reduction of nitrate in the spray. Control spray concentration was 0.43 $\mu eq \cdot L^{-1}$ NO_3–N; experimental spray concentration was 560 $\mu eq \cdot L^{-1}$ NO_3–N.

	Oligotrophic control	Minerotrophic experimental	Oligotrophic experimental
Sphagnum fuscum	91.7	97.5	99.7
Sphagnum magellanicum	37.5	100	99.2

TABLE 5. Nitrate content of in situ *Sphagnum angustifolium* in $\mu g \cdot L^{-1}$ before and after HNO_3 and H_2SO_4 spray application.

	Oligotrophic control		Minerotrophic experimental		Oligotrophic experimental	
Sample #	Pool water	Plant squeezings	Pool water	Plant squeezings	Pool water	Plant squeezings
Concentration of NO_3 $\mu g \cdot L^{-1}$ before spray						
1	1	66	<1	54	<1	25
2	1	44	<1	58	<1	27
3	12	46	2	52	<1	45
Mean ± SE	4.7±4	52±7	<1±0.7	55±2	<1±0	32±6
Concentration of NO_3 $\mu g \cdot L^{-1}$ after spray						
1	<1	138	50	141	71	870
2	<1	101	318	1 720	83	3 340
3	12	143	108	1 620	164	1 290
Mean ± SE	4.0±4	127±13	159±81	1 160±510	106±29	1 833±763
Change in conc. of NO_3 in %	0	240	15 900	2 150	10 600	5 700

of organic anions (Kilham 1982; Hemond 1980). *Sphagnum* spp. may have the capacity to absorb NO_3^- and $SO_4^=$ for some time before becoming "saturated." The ion exchange capacity of *Sphagnum* is well documented (Clymo 1963). As far as we know, no studies have been made to determine the absorption capacity of *Sphagnum* for NO_3^- and $SO_4^=$. Some forest soils have a large capacity to absorb sulfate (Swank et al. 1984) and biologically transform sulfate to organically bound forms (Fitzgerald et al. 1982). In addition, there is evidence that organically bound sulfur in forest soil can be remineralized to inorganic forms (Johnson et al. 1982). If a similar absorption is present in the surface layers of peatland soils, it could account for the disappearance of sulfate from experimental pools.

In British experiments, high concentrations of bisulphite, SO_2, and sulfate have been shown to reduce growth of *Sphagnum* species in both field and laboratory situations (Ferguson et al. 1978; Ferguson and Lee 1980). However recent evidence (Cape 1984) indicates that the aqueous forms of bisulphite/sulfite tested in the British experiments may in fact be SO_2 gas, and therefore the *Sphagnum* may be more susceptible to low concentrations of SO_2 than to sulfate or sulphite. Our results have shown 0.92 $meq \cdot L^{-1}$ of sulfate are not detrimental to *Sphagnum* growth. These values are higher than the average concentrations of $SO_4^=$ in precipitation in eastern Canada. Ferguson et al. 1978 have shown that 10 $meq \cdot L^{-1}$ of sulfate are detrimental to *Sphagnum* growth, and peak precipitation concentrations of 10 $meq \cdot L^{-1}$ $SO_4^=$ have been observed in Brussels (Meurrens 1974, cited in Ferguson et al. 1978).

High NO_3^- and/or ammonium concentrations have also been suggested as being detrimental to *Sphagnum* growth (Press and Lee 1982). In British experiments *Sphagnum* transplanted from relatively unpolluted to polluted areas initially took up NO_3^- rapidly (as measured by nitrate reductase activity) and after 4 d nitrate reductase activity was reduced. Press and Lee (1982) proposed that the decrease in NO_3^- reductase activity (or decrease of NO_3^- uptake) was due to a build up of ammonium in plant tissue which decreased or inhibited nitrate uptake. Again, our results showed that NO_3^- was taken up by the *Sphagnum* both in the laboratory and in the fen. There was no ammonium increase in the adjacent water although tissue concentrations of ammonium were not measured. However, the concentrations of NO_3^- applied experimentally (0.52 $meq \cdot L^{-1}$) were only one half those used in the experiments by Press and Lee (1982) (1 $meq \cdot L^{-1}$), but were close to the average concentrations found in precipitation in eastern Canada (0.56 $meq \cdot L^{-1}$).

In summary, our experiments indicate that on the basis of 1 1/2 yr of addition of H_2SO_4 and HNO_3, the poor fen is a sink for $SO_4^=$ and NO_3^-. The initial response to the addition of strong acids is a temporary increase in concentration of $SO_4^=$ and NO_3^-, followed by a rapid reduction in concentration. Assimilatory NO_3^- reduction and absorption by *Sphagnum* are probably important mechanisms in sequestering NO_3^- which is normally very low in poor fens and bogs. Sulfate assimilation, microbial $SO_4^=$ reduction and H_2S production occur after acidification, even in the oligotrophic areas of the fen at pH 4. All of these reduction mechanisms generate alkalinity in this naturally acid environment. Continued experimental acidification is necessary to determine if the fen vegetation and peat are benefited by the acids and continue to be a sink for NO_3^- and

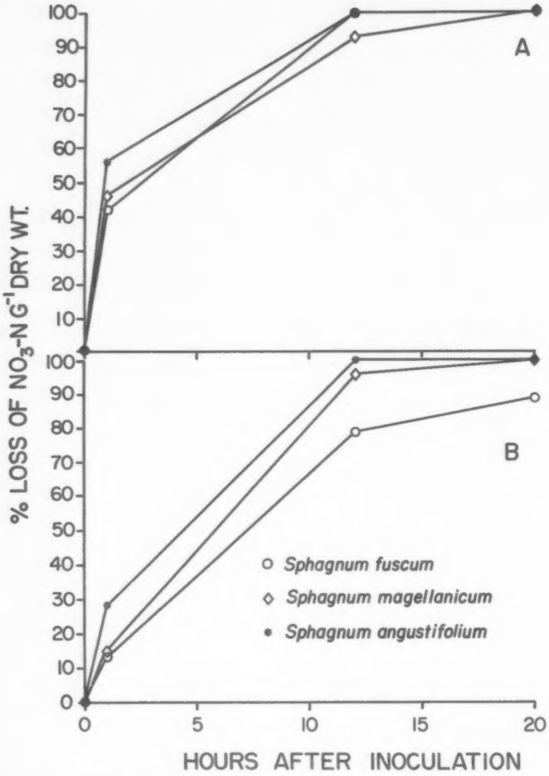

FIG. 10. (A) Removal of NO_3−N from water containing live green *Sphagnum*: (B) Removal of NO_3−N from water containing brown stems of *Sphagnum*.

$SO_4^=$, or if the fen will become saturated and the NO_3^- and $SO_4^=$ will be detrimental to fen vegetation and chemistry.

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Atmospheric Deposition of Nutrients and Major Ions at the Experimental Lakes Area in Northwestern Ontario, 1970 to 1982

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Up to 24 chemical parameters have been monitored in individual precipitation events captured in bulk collectors at the Experimental Lakes Area (ELA), from 1970 to 1982. Although sample to sample variation was high, seasonal trends were discernible for several ions. Annual mean concentrations of H^+, NO_3^-, and SO_4^{2-} were low and quite constant until 1981, but increased dramatically in 1982. Hydrogen ion concentration increased by an average of 0.63 $\mu Eq \cdot L^{-1}$ over this period. The order of annual average cation dominance was $Ca^{2+} > NH_4^+ > H^+ > Mg^{2+} > K^+$. The dominant anion was SO_4^{2-}, followed by $NO_3^- > Cl^-$. Calcium, Mg^{2+}, SO_4^{2-}, Si, suspended P and N, total dissolved P and N, and total ionic concentrations were greatest in spring and fall, correlating with periods of maximum agricultural activity on the prairies, over 250 km away. Concentrations of NO_3^- and H^+ were higher in snow than in rain, while SO_4^{2-}, Ca^{2+}, Mg^{2+}, and NH_4^+ were greater in rain. The total deposition of most substances was higher in summer than in winter, because of the greater proportion of annual precipitation occurring in summer months. A comparison with other sites revealed higher Ca^{2+} and NH_4^+ at all sites near agricultural areas, and higher H^+, SO_4^{2-}, and NO_3^- at sites in eastern North America. A comparison of 1980 bulk versus wet-only chemistry indicated that dryfall contributed 52% of the total annual deposition of Cl^-, 22% of the SO_4^{2-}, 0% of the NO_3^-, 48% of the Mg^{2+}, 31% of the NH_4^+, 28% of the Ca^{2+}, and 8% of the Na^+. Charge balances were excellent for both wet and bulk samples.

Entre 1970 et 1982, on a surveillé jusqu'à 24 paramètres chimiques lors de précipitations individuelles recueillies dans des collecteurs en vrac dans la Région de lacs expérimententaux (RLE). Bien que la variation d'un échantillon à l'autre fusse élevée, des tendances saisonnières étaient discernables pour plusieurs ions. Les concentrations moyennes annuelles de H^+, NO_3^- et de SO_4^{2-} étaient faibles et assez constantes jusqu'à 1981, mais elles ont augmenté de façon spectaculaire en 1982. La concentration des ions hydrogène a augmenté en moyenne de 0,63 $\mu Eq \cdot L^{-1}$ au cours de cette période. L'ordre de dominance pour ce qui est des concentrations moyennes annuelles des cations était le suivant : $Ca^{2+} > NH_4^+ > H^+ < Mg^{2+} > K^+$. L'anion dominant était SO_4^{2-} suivi de $NO_3^- > Cl^-$. Les concentrations de Ca^{2+}, de Mg^{2+}, de SO_4^{2-}, de Si, de P et de N en suspension, de P et N total dissous et les concentrations ioniques totales étaient les plus élevées au printemps et à l'automne, correspondant à des périodes d'activité agricole maximale dans les prairies, jusqu'à 250 km de distance. Les concentrations de NO_3^- et de H^+ étaient plus élevées dans la neige que dans la pluie bien que celles de SO_4^{2+}, de Ca^{2+}, de Mg^{2+} et de NH_4^+ étaient plus fortes dans la pluie. Le dépôt total de la plupart des substances était plus élevé en été qu'en hiver, en raison de la plus forte proportion de précipitations annuelles pendant les mois d'été. Une comparaison faite avec d'autres emplacements a révélé des concentrations plus élevées de la Ca^{2+} et de NH_4^+ à tous les emplacements près des zones agricoles et des concentrations plus fortes de H^+, de SO_4^{2-} et de NO_3^- aux emplacements situés dans l'est de l'Amérique du Nord. Une comparaison de la chimie des substances en vrac par rapport aux substances humides seulement a montré que les retombées sèches représentaient 52 % du dépôt total annuel de Cl^-, 22 % du SO_4^{2-}, 0 % du NO_3^-, 48 % du Mg^{2+}, 31 % du NH_4^+, 28 % du Ca^{2+} et 8 % du Na^+. Les équilibres de charge étaient excellents aussi bien pour les échantillons humides que pour les échantillons en vrac.

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Although recent interest in the acid precipitation problem has led to the establishment of country-wide networks for precipitation monitoring in the United States and Canada, long-term precipitation monitoring using similar collection and analytical methods has been rare. In North America, only Hubbard Brook Experimental Forest has published records which extend back into the 1960's (Likens et al. 1984). These records have proved remarkably valuable in assessing the effects of changes in acidifying emissions on the acidity of precipitation in the eastern USA, and have also illustrated the great year-to-year and month-to-month variation to be expected in precipitation chemistry.

This paper summarizes bulk precipitation records for the Experimental Lakes Area (ELA) from 1970 to 1982. The same collection procedures were used throughout, and the few changes in analytical procedures were carefully intercalibrated. Analyses for unstable compounds were performed on site, usually on the same day of sample collection. Because ELA is a low deposition area, these records provide a unique opportunity to analyze seasonal and long-term trends in various chemicals, in an area where industrial activity is minimal.

Methods

Bulk rain samples were collected at ELA using a 0.25 m^2 acrylic collector to funnel the sample into a 20-L polyethylene carboy. The carboy was changed following each event or at intervals of 24 h during continuous rainfall. Although a polyethylene screen was placed over the collector to exclude debris, occasional samples contained insects or bird droppings and were discarded. The collector was located on a small island in ELA Lake 239 for the entire period of this study. During winter, snow samples were gathered on a similar 0.25 m^2 acrylic collector, equipped with a polyethylene bag instead of a carboy, located at our meteorological site (Atmospheric Environment Service designation: Rawson Lake Station) less than 1 km west of the rain collector. These samples were kept frozen in polyethylene bags for monthly analyses. The amounts of rainfall and water equivalents of snowfall were measured at the meteorological site using Canadian standard rain gauges and shielded Nipher snow collectors. Beaty (1981) provides details of recording procedures and tables of precipitation amounts for individual events.

Immediately following collection of rain or melting of snow, samples were analysed at the ELA laboratory for nitrate nitrogen (NO_3-N), ammonia nitrogen (NH_3-N), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved inorganic carbon (DIC = dissolved $CO_2 + H_2CO_3 + HCO_3^-$), pH, and specific conductance. After the appropriate pre-treatment and preservation (Stainton et al. 1977), samples were submitted to the analytical unit at the Freshwater Institute in Winnipeg, Manitoba for the analysis of suspended carbon, nitrogen and phosphorus, dissolved organic carbon (DOC), soluble reactive silica (SRSi), chloride, sulfate, sodium, potassium, calcium, magnesium, iron, and manganese.

All analytical procedures except for DIC after 1980 are outlined by Stainton et al. (1977). Prokopowich (1979) and Linsey and Braund (1984) provide details of the methods used at ELA during various years. Beginning in 1980, DIC was determined using an infra-red gas analyzer to measure the CO_2 gas evolved from an acidified sample (see Herczeg and Hesslein 1984 for details of this method). Any changes in chemical methods were preceded by extensive intercalibration periods.

Concentrations of substances in ELA bulk precipitation were volume-weighted by the amounts of precipitation recorded at the meteorological site. Hydrogen ion concentrations were calculated by conversion from pH measurements. Perhaps most importantly, considerable effort was devoted to maintaining the integrity of our precipitation monitoring program. One of us (D.W.S.) supervised the program during its entirety. Another (M.P.S.) supervised all chemical analyses performed at the Freshwater Institute. Only two chemists (G.A.L. and J. Prokopowich) supervised the chemical analyses performed at ELA during the period.

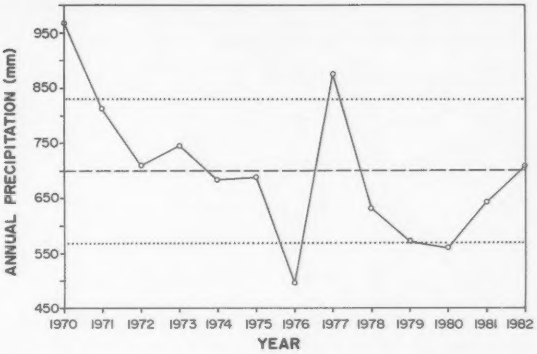

FIG. 1. Annual total amounts of precipitation received at ELA including the 13-yr mean (dashed line) and ±1 standard deviation (dotted lines).

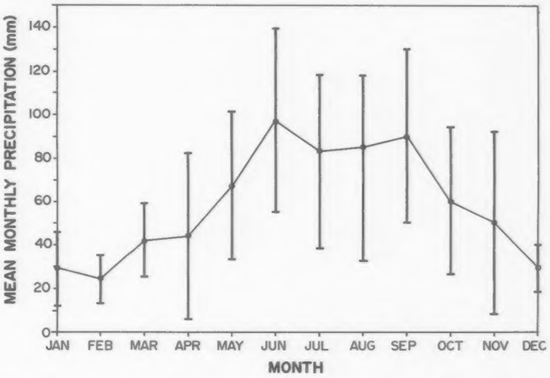

FIG. 2. Mean monthly amounts of precipitation received at ELA, 1970–83. Error bars represent ±1 standard deviation.

Results

1. Amount and Seasonal Distribution of Precipitation

The mean annual amount of precipitation recorded at ELA for the 13-yr period from 1970 to 1982 was 700 mm (Beaty 1981 and unpublished data) with a standard deviation of ±130 mm. This was very similar to the 705 mm average calculated for the 68-yr period of record at Kenora, 50 km northwest of ELA (Newbury and Beaty 1980). Maximum annual precipitation (967 mm) occurred in 1970 and minimum precipitation (496 mm) occurred in 1976 (Fig. 1). The large annual range and coefficient of variation (20%) emphasize the need for a long-term data base when examining and interpreting hydrologic budgets.

The majority of precipitation which occurred from November to March was in the form of snow. In April and October, the proportions of rain and snow varied greatly from year to year. During the remaining months precipitation was almost exclusively rain. Although the amount of precipitation received during any given month varied greatly over the 13-yr period, a marked seasonal distribution was evident (Fig. 2). The winter months of December to February received the least precipitation with the February average being lowest ($\bar{x}$ =

TABLE 1. Annual mean volume-weighted concentrations of various substances in ELA bulk precipitation 1970–1982. Values are in micromoles per litre, unless specified otherwise.

	Year														±1
Substance	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	$\bar{x}$	Standard Deviation
NO_3^--N	16.1	15.0	23.9	15.4	16.5	20.9	16.4	12.6	14.1	20.9	14.6	13.5	28.9	17.6	4.7
NH_4^+-N	16.3	16.3	33.1	15.1	16.5	17.8	20.7	12.4	16.7	22.7	18.4	16.8	40.7	20.3	7.9
TDN	29.6	51.6	61.0	33.9	42.1	49.9	50.9	34.8	42.1	52.4	49.8	45.2	88.0	48.5	15.4
Susp N	12.6	7.6	6.3	10.7	8.3	10.1	12.1	10.9	9.4	14.5	8.3	7.4	4.6	9.5	2.7
ΣN	42.2	59.2	68.0	44.6	50.4	60.0	63.0	45.7	51.5	66.9	58.1	52.6	92.6	58.1	13.3
TDP	0.3	1.4	0.5	0.6	0.9	0.4	0.3	0.3	0.7	0.4	0.2	0.3	0.4	0.5	0.3
Susp P	0.5	0.5	0.4	0.7	0.5	0.5	0.6	0.4	0.5	0.9	0.6	0.5	0.2	0.5	0.2
ΣP	0.8	1.9	0.9	1.3	1.4	0.9	0.9	0.7	1.2	1.3	0.8	0.8	0.6	1.0	0.4
DIC	3.1	—	—	2.7	3.9	1.9	2.2	1.9	2.9	3.0	2.1	2.8	2.6	2.6	0.6
DOC	—	276.8	191.3	462.2	306.6	417.8	333.5	249.9	277.2	264.6	353.8	373.4	570.0	339.8	105.4
Susp C	114.2	123.6	97.0	331.2	176.9	215.2	223.3	176.3	177.8	230.4	148.3	129.3	104.1	172.9	65.6
ΣC	—	—	—	796.1	487.4	634.9	558.9	428.1	457.9	497.0	504.2	505.5	676.7	554.7	114.4
SRSi	—	—	0.5	1.6	1.4	1.0	1.3	1.2	1.7	0.8	1.1	1.1	1.5	1.2	0.4
Cl^-	—	7.7	6.2	8.0	18.4	17.7	15.0	13.3	11.4	14.6	11.0	7.4	21.0	12.6	4.8
SO_4^{2-}	—	—	13.3	14.9	23.0	21.1	13.1	8.9	10.9	15.8	15.3	17.9	30.7	16.8	6.2
Na^+	8.5	9.8	11.7	7.7	8.8	7.9	5.7	3.3	10.9	12.3	8.5	5.5	10.4	8.6	2.6
K^+	3.4	3.6	2.5	—	2.1	1.9	1.7	0.7	1.1	1.3	1.3	1.7	3.8	2.1	1.0
Ca^{2+}	11.6	12.1	10.3	—	11.3	16.1	16.1	4.5	8.3	10.2	10.9	12.6	21.4	12.1	4.3
Mg^{2+}	3.3	5.5	5.9	—	3.8	4.5	5.8	1.4	2.9	3.3	3.8	7.8	5.7	4.5	1.7
Fe	—	—	—	—	—	0.7	0.5	0.9	0.6	1.4	0.8	0.7	0.7	0.8	0.2
Mn	—	—	—	—	—	0.5	0.3	0.2	0.3	0.3	0.2	0.3	0.7	0.4	0.2
H^+	—	—	—	9.0	10.5	12.8	10.4	13.2	10.1	13.3	11.3	12.2	19.3	12.2	2.9
pH	—	—	—	5.05	4.98	4.89	4.98	4.88	5.00	4.88	4.95	4.91	4.72	4.92	0.09
Cond ($\mu S \cdot cm^{-1}$)	13.4	—	—	11.2	16.3	14.9	12.4	11.7	12.9	14.1	11.9	13.6	23.7	14.2	3.5
Amount (mm)[a]	967	811	709	745	683	688	496	874	631	572	560	643	708	700	130

[a]From Beaty (1981) and unpublished data.

24 mm, standard deviation = ± 11 mm). The summer months of June to September received approximately 3 times more precipitation than winter, with June, on average, being the wettest month ($\bar{x}$ = 97 mm, standard deviation = ±42 mm). Roughly 50% of the total yearly precipitation input occurred during the four months from June to September and about 30% of the yearly total was received as snow.

2. Annual Mean Concentrations and Deposition

The weighted annual mean concentrations of most substances (Table 1) had coefficients of variation of from 25 to 40% of their 13-yr means. Exceptions were TDP, Mn, and K^+ which were even greater (C.V. = 63%, 45%, and 49%, respectively). Hydrogen ion had the lowest coefficient of variation (23%). Due to the large year-to-year variations which represented ranges of up to 6 times between maximum and minimum annual values, it was not possible to recognize statistically-significant trends in concentration over time for any substance measured except H^+ which increased by an average of 0.63 $\mu Eq \cdot L^{-1} \cdot yr^{-1}$ ($P < 0.05$). Although NO_3^-, SO_4^{2-}, and Cl^- concentrations did not change significantly with time, all were positively correlated with H^+ concentration (NO_3^-: $r = 0.79$, $P < 0.01$, SO_4^{2-}: $r = 0.65$, $P < 0.05$, Cl^-: $r = 0.60$, $P < 0.05$).

TABLE 2. Weighted annual mean concentrations of total major cations and anions in ELA bulk precipitation 1970–82.

Year	Σ Cations ($\mu Eq \cdot L^{-1}$)	Σ Anions ($\mu Eq \cdot L^{-1}$)	Cations−Anions	Unmeasured cations or anions as % of total ions
1970	—[a]	—	—	—
1971	—	—	—	—
1972	—	56.54	—	—
1973	—	53.16	—	—
1974	67.91	80.96	−13.05	−8.8
1975	81.24	80.89	0.35	0.2
1976	82.02	57.42	24.60	17.6
1977	41.50	43.68	−2.18	−2.6
1978	58.37	47.29	11.08	10.5
1979	75.06	66.99	8.07	5.7
1980	72.53	56.28	16.25	12.6
1981	76.87	56.67	20.20	15.1
1982	129.95	111.31	16.64	7.0

[a]One or more major ions not measured during that year.

To check the completeness of our analyses, a charge balance was estimated using the annual mean equivalent concentrations of measured ions (Table 2). For seven of the nine years when all major ions were measured, cations exceeded anions by an average of 14 $\mu Eq \cdot L^{-1}$. Unmeasured anions could have included HCO_3^- and PO_4^{3-}, but judging from the pH of our precipitation and values available for a site 200 km south of ELA (Verry 1983), these would have only contributed up to 2 $\mu Eq \cdot L^{-1}$. Another ion which was excluded from our calculations was F^-, which was found in concentrations of from 2 to 5 $\mu Eq \cdot L^{-1}$ in bulk precipitation in southern Ontario (Scheider et al. 1979). The same study suggested that negatively charged oxides of Mn, Fe, and Si originating from soils could have contributed to unmeasured anions. This seems possible for ELA because it is close to (250 km) and predominantly downwind from cultivated prairie areas.

The concentrations of both total cations and anions remained relatively constant between 40 and 80 $\mu Eq \cdot L^{-1}$ until 1982, when both values almost doubled (Table 2), despite an average amount of precipitation (708 mm) in that year. The high ion concentrations in 1982 resulted from large increases in the

TABLE 3. Correlation coefficients (R) of equivalent concentrations of ion pairs in individual ELA bulk precipitation events.

Species combination	n	prob>|R|	Correlation coefficient (r)	Species combination	n	prob>|R|	Correlation coefficient (r)
NH_4:SO_4	617	0.0001	0.54011	H:SO_4	515	0.0001	0.36576
NH_4:NO_3	722	0.0001	0.19069	H:NO_3	543	0.0001	0.34743
NH_4:Cl	624	0.0354	0.08422	H:Cl	506	0.0004	0.15593
NH_4:amount of ppt	717	0.1902	−0.04897	H:amount	557	0.9630	0.00197
Ca:SO_4	530	0.0001	0.64708	Mg:SO_4	530	0.0001	0.4757
Ca:NO_3	602	0.0001	0.50661	Mg:NO_3	602	0.0001	0.38511
Ca:Cl	537	0.0001	0.25233	Mg:Cl	537	0.0001	0.17678
Ca:amount	606	0.0001	−0.20328	Mg:amount	606	0.0004	0.14461
Na:SO_4	608	0.0001	0.17589	K:SO_4	529	0.0001	0.45880
Na:NO_3	687	0.0001	0.18252	K:NO_3	601	0.0001	0.28734
Na:Cl	615	0.3092	0.04108	K:Cl	536	0.0001	0.24193
Na:amount	691	0.0073	−0.10192	K:amount	605	0.0021	0.12476
				SO_4:amount	622	0.0001	−0.22050
				NO_3:amount	717	0.0001	−0.15213
				Cl:amount	629	0.0705	−0.07217

TABLE 4. Total annual deposition of substances from ELA bulk precipitation, 1970–82. Values are in millimoles per square metre.

	Year														±1
Substance	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	$\bar{x}$	Standard deviation
NO_3^--N	15.54	12.17	16.91	11.66	11.27	14.39	8.11	10.98	8.88	11.96	8.16	8.68	20.42	12.52	3.52
NH_4^+-N	16.03	13.21	23.33	11.44	11.27	12.23	10.27	10.86	10.55	23.98	10.38	10.79	28.81	14.0	5.71
TDN	28.67	41.90	43.24	25.59	28.73	34.34	25.25	30.40	26.61	29.96	27.88	29.05	62.27	33.38	10.37
Susp N	12.23	6.20	4.46	8.10	5.66	6.93	6.02	9.55	5.91	8.29	4.64	4.77	3.29	6.62	2.42
ΣN	40.70	48.10	47.70	33.69	34.39	41.27	31.27	40.95	35.52	38.25	32.52	33.82	65.56	40.06	9.49
TDP	0.32	1.13	0.36	0.48	0.61	0.26	0.13	0.29	0.48	0.23	0.13	0.19	0.26	0.37	0.27
Susp P	0.48	0.39	0.29	0.55	0.36	0.36	0.29	0.36	0.36	0.52	0.36	0.29	0.16	0.37	0.10
ΣP	0.80	1.52	0.65	1.03	1.97	0.62	0.42	0.65	0.84	0.75	0.49	0.48	0.42	0.82	0.46
DIC	39.74	—	—	27.27	35.91	17.37	14.42	22.36	24.84	15.05	15.54	24.10	24.59	23.74	8.31
DOC	—	224.53	135.58	349.26	209.40	287.39	165.35	218.43	175.00	151.21	198.00	239.86	403.33	229.78	80.68
Susp C	110.42	100.26	58.75	250.26	120.83	147.99	110.69	154.12	112.23	131.68	83.05	83.11	73.65	119.00	47.58
ΣC	—	—	—	626.79	366.14	452.75	290.46	394.91	312.07	297.94	296.59	347.07	501.57	372.52	136.57
SRSi	—	—	0.39	1.18	0.93	0.68	0.64	1.03	1.07	0.46	0.61	0.68	1.07	0.79	0.27
Cl^-	—	6.26	4.37	6.06	12.56	12.15	7.42	11.62	7.22	8.32	6.18	4.80	14.85	8.48	3.44
SO_4^{2-}	—	—	9.39	11.24	15.74	14.54	6.47	7.77	6.91	9.00	8.59	11.48	21.75	11.17	4.59
Na^+	8.26	7.96	8.31	5.87	6.00	5.39	2.78	2.91	5.18	6.22	4.77	3.57	7.35	5.90	1.91
K^+	3.32	2.89	1.76	—	1.46	1.33	0.84	0.61	0.73	0.72	0.69	1.07	2.66	1.51	0.95
Ca^{2+}	11.14	9.78	7.31	—	7.68	11.01	7.93	3.92	5.19	5.84	6.11	8.06	15.07	8.25	3.08
Mg^{2+}	3.21	4.44	4.20	—	2.59	3.08	2.88	1.28	1.85	1.89	2.14	5.02	4.03	3.05	1.17
Fe	—	—	—	—	—	0.50	0.27	0.77	0.41	0.77	0.43	0.47	0.50	0.52	0.17
Mn	—	—	—	—	—	0.33	0.13	0.18	0.20	0.20	0.13	0.22	0.51	0.24	0.13
H^+	—	—	—	6.77	7.15	8.78	5.15	11.55	6.38	7.60	6.33	7.87	13.64	8.12	2.60

NO_3^-, NH_4^+, H^+, SO_4^{2-}, Ca^{2+}, K^+, and Cl^- values. Concentrations of all other substances remained constant.

The distribution of anions on an equivalent basis in ELA bulk precipitation ($SO_4^{2-} > NO_3^- > Cl^-$) was the same as that reported in other areas underlain by the Precambrian Shield, where acidic deposition was much higher (e.g. Beamish and Van Loon 1977; Scheider et al. 1979). However, at ELA the cations were dominated by Ca^{2+} and NH_4^+ followed by $H^+ > Mg^{2+} > K^+$. This was unlike more easterly locations where H^+ was the dominant cation (Likens et al. 1977, 1984; Scheider et al. 1979).

A correlation matrix of concentrations of cations versus anions for individual events was constructed to determine which ion species combinations were prevalent in ELA bulk precipitation. The amount of precipitation recorded for each event was included in this exercise (Table 3). The highest correlations were found between Ca^{2+} and SO_4^{2-}, NH_4^+ and

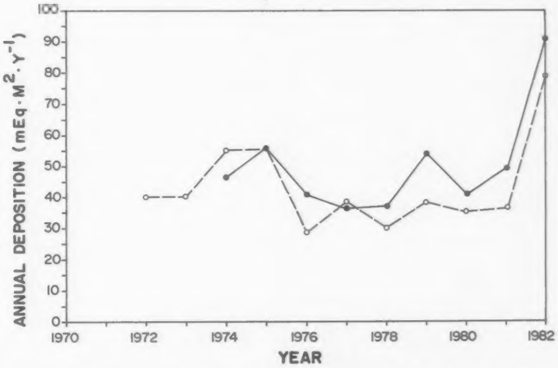

FIG. 3. Annual deposition of total cations (solid line) and anions (dashed line) in ELA bulk precipitation, 1970–82.

TABLE 5. Average weighted monthly mean concentrations of substances in ELA bulk precipitation, 1970–82. The figures in brackets indicate the number of years for which data were collected. Values are in micromoles per litre unless specified otherwise.

	Month											
Substance	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
NO_3^--N	28.1 (10)	27.6 (12)	21.5 (11)	27.4 (4)	24.5 (13)	17.3 (13)	14.9 (13)	12.8 (13)	16.9 (13)	13.9 (13)	18.3 (11)	24.6 (12)
NH_4^+-N	15.6 (10)	21.2 (12)	25.2 (11)	40.4 (4)	36.1 (13)	23.5 (13)	14.5 (13)	16.0 (13)	26.9 (13)	18.3 (13)	13.0 (11)	16.1 (12)
TDN	47.5 (10)	53.6 (12)	35.6 (11)	53.8 (4)	75.8 (13)	66.1 (13)	45.1 (13)	36.5 (13)	52.7 (13)	41.9 (13)	33.8 (11)	49.1 (12)
Susp N	7.5 (9)	7.4 (10)	5.4 (9)	5.5 (4)	19.1 (13)	13.8 (12)	16.7 (12)	9.1 (12)	7.7 (12)	8.4 (13)	7.6 (11)	5.9 (10)
ΣN	54.9 (9)	61.0 (10)	41.0 (9)	59.3 (4)	94.9 (13)	79.9 (12)	61.8 (12)	45.6 (12)	60.4 (12)	50.3 (13)	41.4 (11)	55.0 (10)
TDP	0.3 (10)	0.3 (12)	0.5 (11)	1.0 (4)	0.7 (3)	0.8 (13)	0.7 (13)	0.2 (13)	0.3 (13)	0.2 (13)	0.3 (11)	0.3 (12)
Susp P	0.2 (9)	0.2 (10)	0.3 (4)	0.2 (4)	1.6 (13)	1.1 (12)	0.9 (12)	0.3 (12)	0.4 (12)	0.4 (13)	0.3 (11)	0.1 (11)
ΣP	0.5 (9)	0.5 (10)	0.7 (10)	1.2 (4)	2.3 (13)	1.9 (12)	1.6 (12)	0.5 (12)	0.7 (12)	0.6 (13)	0.6 (11)	0.4 (11)
DIC	2.3 (6)	2.0 (7)	2.9 (7)	4.7 (3)	3.1 (11)	2.8 (11)	3.0 (11)	2.3 (11)	2.5 (11)	2.7 (11)	2.4 (4)	2.0 (6)
DOC	268.3 (7)	357.7 (9)	451.8 (10)	329.8 (4)	414.7 (11)	339.7 (11)	440.5 (11)	311.8 (11)	287.3 (11)	382.5 (11)	234.1 (11)	295.6 (10)
Susp C	114.0 (9)	131.1 (11)	93.3 (10)	106.8 (4)	262.0 (13)	273.6 (12)	196.9 (12)	146.3 (12)	141.6 (12)	179.5 (13)	87.3 (11)	110.6 (10)
ΣC	384.6 (6)	508.8 (7)	548.0 (7)	441.3 (3)	679.8 (11)	616.1 (11)	640.4 (11)	460.4 (11)	431.4 (11)	564.7 (11)	328.8 (4)	408.2 (6)
SRSi	0.6 (4)	0.5 (6)	0.5 (5)	4.1 (3)	1.4 (11)	1.2 (12)	1.5 (11)	1.3 (12)	1.2 (11)	1.2 (11)	1.2 (5)	0.7 (5)
Cl^-	13.5 (9)	14.0 (11)	15.9 (9)	10.6 (4)	14.0 (12)	12.4 (12)	14.3 (12)	15.1 (12)	13.1 (12)	15.7 (12)	13.6 (10)	6.9 (12)
SO_4^{2-}	7.7 (9)	12.5 (11)	23.6 (9)	25.6 (4)	32.3 (11)	19.8 (11)	15.8 (11)	17.7 (12)	14.5 (12)	23.1 (12)	10.4 (10)	8.9 (11)
Na^+	8.6 (10)	10.2 (11)	9.4 (9)	10.7 (4)	6.7 (12)	6.7 (12)	8.5 (12)	7.1 (12)	7.7 (12)	12.3 (12)	11.5 (10)	9.1 (11)
K^+	1.6 (10)	1.2 (11)	1.6 (9)	2.4 (4)	2.3 (12)	2.7 (12)	3.1 (12)	2.0 (12)	1.9 (12)	1.5 (12)	2.1 (10)	1.6 (11)
Ca^{2+}	10.9 (10)	6.5 (11)	7.8 (9)	22.6 (4)	14.7 (12)	11.9 (12)	17.5 (12)	12.0 (12)	20.2 (12)	11.6 (12)	11.6 (10)	8.1 (11)
Mg^{2+}	3.1 (10)	1.9 (11)	2.9 (9)	5.1 (4)	5.2 (12)	5.8 (12)	7.4 (12)	5.0 (12)	5.9 (12)	5.1 (12)	3.1 (10)	2.1 (11)
Fe	0.7 (7)	0.6 (8)	0.5 (6)	0.4 (3)	1.1 (8)	0.7 (8)	0.6 (8)	0.9 (8)	0.7 (8)	0.8 (8)	0.7 (7)	0.7 (8)
Mn	0.3 (6)	0.2 (7)	0.5 (5)	0.4 (3)	0.9 (8)	0.4 (8)	0.3 (8)	0.4 (8)	0.4 (8)	0.3 (8)	0.4 (7)	0.3 (8)
H^+	16.3 (7)	20.7 (8)	15.3 (7)	18.1 (4)	15.6 (9)	10.2 (10)	7.8 (11)	10.8 (11)	8.6 (10)	13.6 (10)	10.2 (8)	21.8 (9)
pH	4.79(7)	4.68(8)	4.82(7)	4.74(4)	4.81(9)	4.99(10)	5.11(11)	4.97(11)	5.06(10)	4.87(10)	4.99(8)	4.66(9)
Cond ($\mu S \cdot cm^{-1}$)	14.1 (8)	15.7 (9)	14.8 (8)	22.7 (3)	16.0 (10)	13.2 (11)	13.9 (11)	12.9 (11)	15.1 (11)	16.2 (11)	10.4 (8)	13.2 (9)
Amount (mm)[a]	28.7	24.4	42.3	44.2	67.3	97.3	83.4	85.0	89.7	60.5	50.2	28.6

[a] From Beaty (1981) and unpublished data.

TABLE 6. Weighted mean concentrations of substances in rain and snow, 1970–82.

	Snow		Rain	
Substance	($\mu g \cdot L^{-1}$)	($\mu Eq \cdot L^{-1}$)	($\mu g \cdot L^{-1}$)	($\mu Eq \cdot L^{-1}$)
NO_3^-	1 489	24.01	1 131	18.25
NH_4^+	328	18.23	452	25.09
Susp N	94		161	
TDN	633		744	
Susp P	6		22	
TDP	10		18	
DIC	375		484	
DOC	3 901		4 296	
Susp C	1 287		2 240	
Si	21		49	
Cl^-	476	13.41	482	13.59
SO_4^{2-}	1 207	25.15	2 039	42.49
Na^+	225	9.78	196	8.53
K^+	63	1.61	89	2.28
Ca^{2+}	360	17.98	633	31.64
Mg^{2+}	64	5.37	138	11.46
Fe	37		42	
Mn	20		24	
H^+	16.85	16.85	12.10	12.10
pH	4.77		4.92	
Amount (mm)[a]	204		493	
Cations		69.82		91.10
Anions		62.57		74.33

[a] From Beaty (1981) and unpublished data.

SO_4^{2-}, Ca^{2+} and NO_3^-, K^+ and SO_4^{2-}, Mg^{2+} and SO_4^{2-}, Mg^{2+} and NO_3^-, H^+ and SO_4^{2-}, and H^+ and NO_3^-. Most major cations and anions showed negative but weak correlations with the amount of precipitation in individual events which indicated a dilution effect in higher volume episodes.

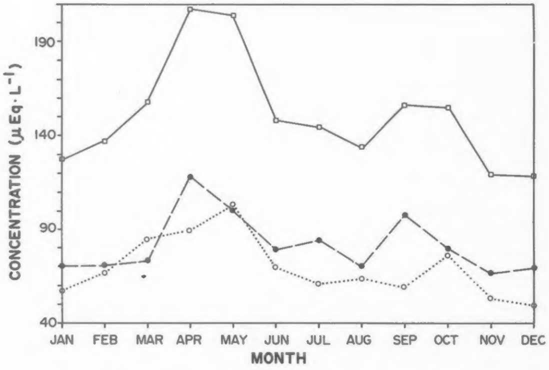

FIG. 4. Weighted, monthly mean concentrations of cations (dashed line) and anions (dotted line) and cations plus anions (solid line) in ELA bulk precipitation, 1970–82.

Trends in annual deposition rates of substances through time were similar to the trends in concentration. The annual bulk deposition of anions and cations at ELA was relatively constant from 1972 to 1981 at 30 to 50 mEq·m^{-2}·yr^{-1}. In 1982, deposition of total cations and anions nearly doubled to values of 91 and 79 mEq·m^{-2}·yr^{-1}, respectively (Fig. 3). Even including the 1982 data, the average annual deposition of major ions at ELA (Table 4) was comparatively low.

3. Seasonal Trends in the Concentration and Deposition of Substances

The maximum monthly mean concentrations of total ions in ELA bulk precipitation occurred in April and May, with a

TABLE 7. Mean total monthly deposition of substances from ELA bulk precipitation, 1970–82. Values are in micromoles per square metre.

	Month											
Substance	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
NO_3^--N	806	673	909	1 209	1 649	1 682	1 234	1 087	1 518	843	918	705
NH_4^--N	447	518	1 067	1 784	2 432	2 287	1 209	1 360	2 409	1 105	653	462
TDN	1 363	1 307	1 505	2 377	5 100	6 436	3 759	3 103	4 728	2 537	1 696	1 405
Susp N	211	180	230	243	1 284	1 341	1 394	771	692	510	692	170
ΣN	1 574	1 487	1 735	2 620	6 384	7 777	5 153	3 874	5 420	3 047	2 080	1 575
TDP	8	6	22	46	46	78	59	19	29	12	16	8
Susp P	6	4	7	7	109	104	75	27	38	23	16	3
ΣP	14	10	29	53	155	182	134	46	67	35	32	11
DIC	899	653	1 646	2 773	2 765	3 608	3 378	2 685	2 990	2 188	1 648	753
DOC	7 699	9 166	19 113	14 579	27 907	33 050	36 738	26 506	25 766	23 141	11 751	8 454
Susp C	3 272	3 198	4 301	4 722	17 633	26 620	15 423	12 431	12 700	10 860	4 384	3 163
ΣC	11 870	13 017	25 060	22 075	48 305	63 278	56 539	41 622	41 456	36 189	17 783	12 370
SRSi	18	17	23	182	96	121	128	109	112	75	63	21
Cl^-	388	342	671	469	940	1 209	1 189	1 281	1 172	949	684	287
SO_4^{2-}	217	306	996	1 129	2 173	1 931	1 314	1 507	1 297	1 396	521	253
Na^+	247	250	397	471	451	656	711	606	690	742	578	261
K^+	46	29	66	105	157	259	262	170	172	91	105	45
Ca^{2+}	314	158	331	999	987	1 662	1 458	1 020	1 814	700	581	230
Mg^{2+}	90	47	124	227	352	561	621	427	528	311	157	61
Fe	21	16	21	19	77	66	54	75	64	46	37	19
Mn	7	15	20	16	61	41	27	34	34	18	18	9
H^+	468	505	645	798	1 051	994	651	915	773	825	514	623

smaller peak in September–October (Fig. 4). This resulted from increased concentrations of the dominant cations (Ca^{2+} and NH_4^+) and anion (SO_4^{2-}) which comprised over 50% of their respective totals (Table 5). Total dissolved N and P, suspended N and P, and suspended C, Fe, Mn, and Si showed similar seasonal patterns. These were periods of maximum soil cultivation on the prairies, and we believe that the above substances were carried to ELA by prevailing winds. Spring pollen deposition may have also contributed to the high C, N, and P values. Banks (1981) observed that pollen comprised a high proportion of annual P input to lakes in eastern Ontario.

Concentrations of H^+ and NO_3^- were highest during winter, and probably originated from populated and industrialized areas to the south. Most other substances including SO_4^{2-} had summer maxima.

A comparison between the mean concentrations of substances found in snow and rain (Table 6) showed that NO_3^- was the only anion that, on average, had a higher concentration in snow. Chloride was similar in rain and snow while SO_4^{2-} concentrations were much higher in rain. Of the cations, only H^+ and Na^+ had higher concentrations in snow. Potassium was similar in rain and snow while Ca^{2+}, Mg^{2+}, and NH_4^+ concentrations were greater in rain. The same pattern was evident at 3 other sites located within a 300 km radius to the south and southwest of ELA: Tewaukon, North Dakota, Itasca, northern Minnesota (Munger 1982); and Marcell Experimental Forest, north-central Minnesota (Verry 1983). Another site, Hovland in northeastern Minnesota (Munger 1982), approximately 300 km southeast of ELA, was similar with the exception of SO_4^{2-} which was much higher in snow.

The dominant cation in ELA snow was NH_4^+ followed closely by $Ca^{2+} > H^+$ and then $Na^+ > Mg^{2+} > K^+$. This closely resembled the distribution observed at Hovland, except H^+ was dominant and the concentration more than 3 times higher than at ELA. In ELA rain, Ca^{2+} was greater than NH_4^+ followed by $H^+ > Mg^{2+} > Na^+ > K^+$, identical to the distribution at Hovland. Concentrations of cations in rain were similar at ELA, Itasca, Hovland, and Marcell while at Tewaukon, located in an agricultural area, the respective values were 2 to 3 times higher.

The distribution of anions in snow was the same at ELA, Marcell, and Hovland ($SO_4^{2-} > NO_3^- > Cl^-$), with the concentrations of each being similar at ELA and Marcell. At Hovland, values for all 3 anions averaged 2 times those at ELA. The same distribution was noted in rain at ELA and Marcell with concentrations of the respective anions being similar at each site. At Hovland, Cl^- was dominant while SO_4^{2-} and NO_3^- concentrations decreased to values similar to ELA and Marcell.

Since H^+ and NO_3^- concentrations in snow were greater than in rain by almost the same amount at ELA (Table 6), it appeared that nitric acid influenced the seasonal variation in acidity of precipitation in this area. Regression analyses of monthly mean concentrations of H^+ versus NO_3^- and SO_4^{2-} showed that NO_3^- could explain 66% of the variation in H^+ while no significant relationship existed between H^+ and SO_4^{2-} ($r^2 = 0.002$). The high equivalent concentration of SO_4^{2-} in rain (almost twice that of snow) must therefore have resulted from its deposition in association with cations other than H^+, primarily the soil-derived cations Ca^{2+}, Mg^{2+}, and K^+ (Table 3).

The average deposition of most substances was greatest during the months of May to October (Table 7) reflecting both the increased concentrations of most ions at that time (Fig. 5) and the much higher amounts of precipitation during those months (Fig. 2). Most substances showed maximum deposition in June which was the month in which the greatest amount of precipitation was generally recorded. However, NH_4^+,

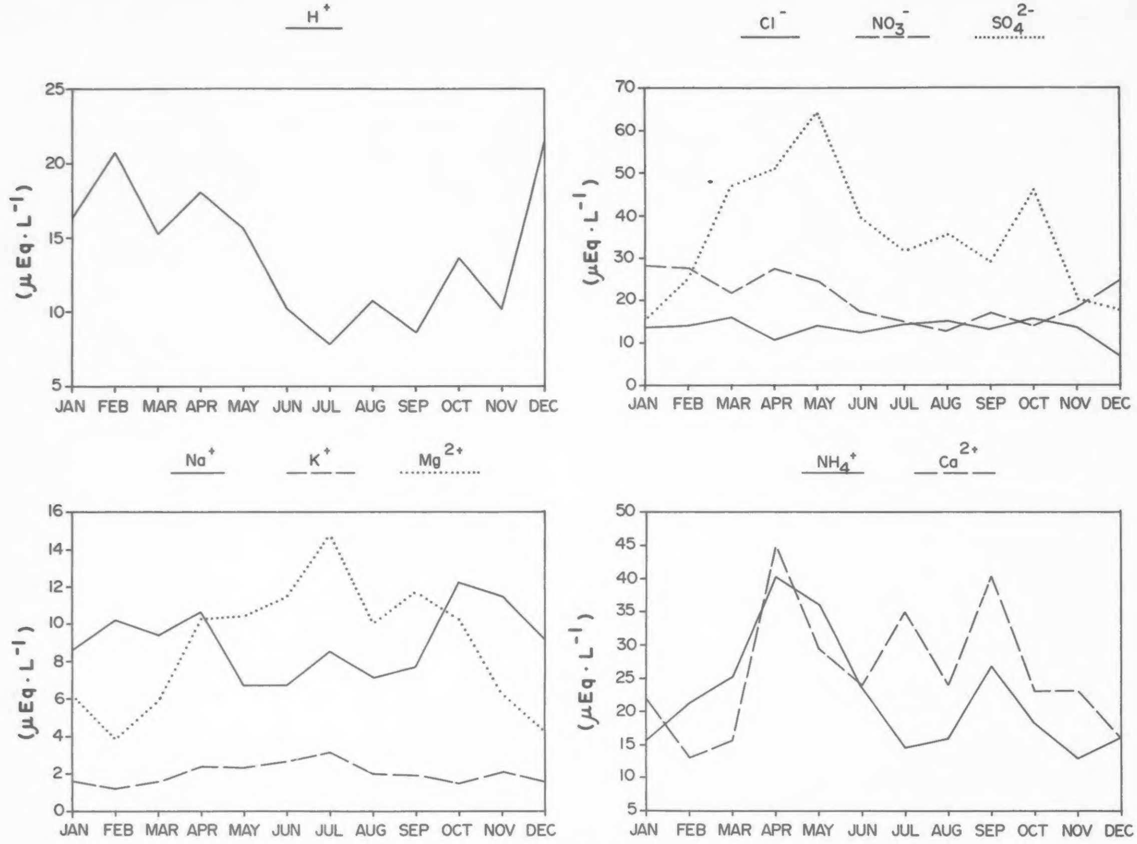

FIG. 5. Weighted mean monthly concentrations of individual ions in ELA bulk precipitation, 1970–82.

TABLE 8. A comparison of the annual mean concentrations and total deposition of major ions in ELA wet-only (from Barrie and Sirois 1982) and bulk precipitation (this study) for 1980.

	Concentration ($\mu M \cdot L^{-1}$)		Deposition ($mM \cdot m^{-2} \cdot yr^{-1}$)	
Substance	Wet	Bulk	Wet[a]	Bulk
SO_4^{2-}	12.0	15.3	6.7	8.6
NO_3^-	14.6	14.6	8.2	8.2
Cl^-	5.2	11.0	3.0	6.2
NH_4^+	12.9	18.4	7.2	10.4
Ca^{2+}	8.3	10.9	4.4	6.1
Mg^{2+}	2.0	3.8	1.1	2.1
K^+	2.7	1.2	1.5	0.7
Na^+	7.9	8.5	4.4	4.8
H^+	9.9	11.3	5.5	6.3
pH	5.0	4.95		
Amount (mm)			509	560

[a] Corrected for undercatch calculated from ELA collectors.

SO_4^{2-}, and H^+ deposition was greatest in May when the amount of precipitation was considerably lower than in the summer months. Silica and Na^+ showed maxima in April and October which were comparatively dry months (Fig. 2). High deposition rates during months in which the amount of precipitation was low indicated the extent to which dry deposition contributed to the input of numerous substances. Similar seasonal deposition patterns were presented by Scheider et al. (1979) for stations in south central Ontario.

4. Bulk Versus Wet-Only Collections

To estimate the amounts of major ions entering our bulk collectors as dry deposition, the annual weighted means calculated from the ELA data were compared to those based upon wet-only event samples collected in 1980 at the ELA meteorological site as part of the CANSAP program (Table 8, Barrie and Sirois 1982). The CANSAP event sampler captured only 91% as much precipitation (509 mm) as was measured at the same location by our standard rain gauge and Nipher shielded snow collectors (560 mm). Much of this difference could have occurred due to the lack of sensitivity to snow of the sensor mechanism which opened the CANSAP collector. Catch inefficiency could also result from aerodynamic flow around the collector (Barrie and Sirois 1982).

Concentrations of SO_4^{2-}, Mg^{2+}, Ca^{2+}, Na^+, NH_4^+, Cl^-, and H^+ were higher in the bulk samples. Nitrate was similar in both, while K^+ was higher in wetfall. The proportion (bulk-wet)/bulk for each of the above was 0.22, 0.48, 0.28, 0.08, 0.31, 0.52, 0.13, 0.00, and −1.14, respectively. These numbers are generally similar to those observed by Likens et al. (1984). Charge balances were excellent for both wet and bulk samples, the anions balancing 96 and 97% of the cations, respectively.

Annual deposition rates of these ions were calculated from wet and bulk samples after adjusting the CANSAP data for sampler undercatch. The proportion of the total 1980 bulk deposition that occurred as dryfall ranged from 0% for NO_3^- to 48% for Mg^{2+}. An estimated 22% of the SO_4^{2-} and 13% of the H^+ could be attributed to dryfall.

Discussion

Although ELA is located in a forested area on the Precambrian Shield, the precipitation chemistry appeared to be influenced to a great extent by dust originating in cultivated prairie areas some 250 km to the west. Both the highest concentrations of major ionic constituents [Ca^{2+}, K^+, and SO_4^{2-} (Fig. 5) plus Si] and the seasonal pattern of deposition of most substances, correlated with periods when prairie soils were not snow covered, and particularly with times of maximum cultivation. Similar trends were apparent in precipitation data from northern Minnesota and North Dakota (Fig. 6; Munger 1982; Verry 1983).

Comparison of ELA data with more remote sites further illustrated this prairie influence. Much lower concentrations of Ca^{2+} and SO_4^{2-} were recorded at Saqvaqjuac, on the western coast of Hudson Bay (Fig. 6), even though the latter station was strongly influenced by marine aerosols, as shown by Na^+ and Cl^- values (H. E. Welch, unpublished data). Deposition of all ions except H^+ was far lower at Poker Flat, Alaska (Galloway et al. 1982) than at ELA. The similarity in H^+ values between ELA, Saqvaqjuac, and Poker Flat suggests that all three had near-background levels of acidity.

In contrast, precipitation from more southern and eastern sites in North America all had much higher concentrations of H^+ than ELA, regardless of other chemical characteristics. The high H^+ values at Dorset, in south-central Ontario, and Hubbard Brook, New Hampshire, were correlated with higher SO_4^{2-} and NO_3^- concentrations than present at ELA. The Dorset site also showed remarkably high levels of base cations, much higher than even the ELA and Minnesota sites (Fig. 6). In contrast, the base cations at Hubbard Brook were far lower than at ELA, Saqvaqjuac, or the Minnesota sites, approaching values for Poker Flat. This, plus a long-term declining trend in concentrations of most cations (Likens et al. 1984), may reflect less agricultural activity and improved control at least of particulate air pollutants in the northeastern U.S.

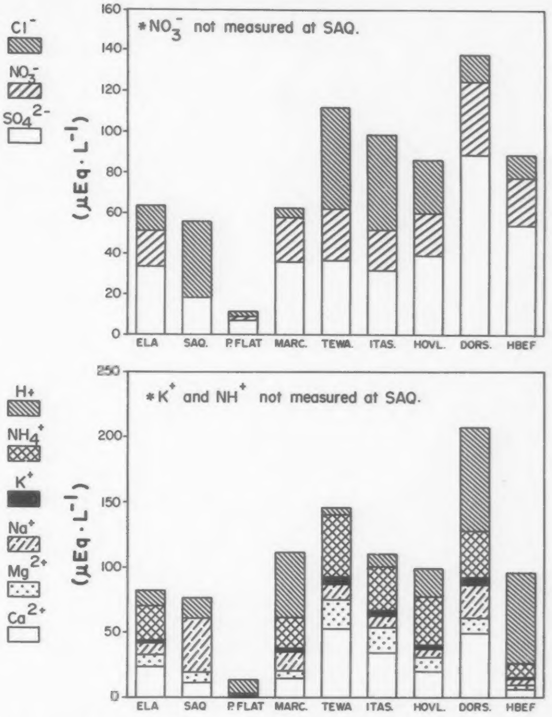

FIG. 6. Weighted mean concentrations of ionic components of precipitation at nine sites in North America. Saqvaqjuac (Northwest Territories, Canada) data from H. E. Welch (unpubl. data), Poker Flat (Alaska, USA) data from Galloway et al. (1982), Marcell (Minnesota, USA) data from Verry (1983), Dorset (Ontario, Canada) data from Scheider et al. (1979), Hubbard Brook Experimental Forest (New Hampshire, USA) data from Likens et al. (1984). Tewaukon (North Dakota, USA), Itasca and Hovland (Minnesota, USA) data from Munger (1982). Note the higher H^+, SO_4^{2-}, and NO_3^- values at sites southeast of ELA (Hovland, Dorset, and Hubbard Brook).

The same seasonal distribution of NO_3^- and H^+, with higher winter values, was common to ELA, the three Minnesota sites, and Tewaukon, North Dakota (Table 9). In addition, the concentrations of these 2 ions at ELA for winter and summer were almost identical to those of Itasca and Marcell which are almost directly south of ELA. The magnitude of the increases in NO_3^- and H^+ from summer to winter were not only consistent between ELA, Marcell, Tewakon, and Itasca, but the amounts by which the winter exceeded the summer concentrations for both ions at each site were almost identical. At Hovland, southeast of ELA, summer NO_3^- and H^+ values approximated those at ELA. However, in winter, NO_3^- and H^+ concentrations at Hovland were almost 2 times higher than ELA and were similar to values reported at Dorset in south-central Ontario. This was also true for SO_4^{2-} which decreased in winter at ELA, Marcell, Itasca, and Tewakon. These data suggest that a steeply increasing gradient in NO_3^-, SO_4^{2-}, and H^+ deposition extended from ELA, southeast through southern Ontario to the northeastern U.S. We believe that the higher winter NO_3^- and H^+ concentrations at ELA reflected an increased incidence of precipitation events which originated over the lower Great Lakes region. Barrie et al. (1980), using back trajectory analyses, concluded that area was in fact the primary source for particulate SO_4^{2-} and gaseous SO_2 deposited at ELA over a short period of time in February 1979. Large increases in the annual mean concentrations of H^+, NO_3^-, SO_4^{2-}, Cl^-, NH_4^+, and Ca^{2+} were observed at ELA in 1982, while the amount of precipitation that year equalled the 13-yr mean. This may have resulted from atmospheric conditions that promoted a greater number of precipitation episodes that originated to the southeast. It would appear that ELA is situated in an area where the precipitation chemistry is influenced not only by the deposition of soil-derived substances from the prairies to the west, but also by long range transport of anthropogenic emissions from the industrialized lower Great Lakes region to the southeast.

The neutralizing effects of prairie dust and NH_4^+ aerosols on

TABLE 9. Mean concentrations of major ions in summer and winter precipitation at the ELA, Marcell (from Verry 1983), Itasca, Hovland, and Tewaukon (from Munger 1982) sites. Values are μEq·L^{-1}.

ELA				Marcell				Hovland				Itasca				Tewaukon			
Summer		Winter		Summer		Winter		Summer		Winter		Summer		Winter		Summer		Winter	
Ca^{2+}	32	NH_4^+	18	NH_4^+	26	H^+	22	Ca^{2+}	24	H^+	58	NH_4^+	31	Mg^{2+}	26	Ca^{2+}	72	Ca^{2+}	32
NH_4^+	25	Ca^{2+}	18	Ca^{2+}	17	Na^+	20	NH_4^+	21	NH_4^+	29	Ca^{2+}	24	Ca^{2+}	25	NH_4^+	49	Mg^{2+}	24
H^+	12	H^+	17	Na^+	16	Ca^{2+}	11	H^+	14	Ca^{2+}	21	H^+	10	H^+	18	Mg^{2+}	22	NH_4^+	18
Mg^{2+}	11	Na^+	10	Mg^{2+}	10	NH_4^+	10	Mg^{2+}	8	Na^+	8	Mg^{2+}	8	NH_4^+	14	Na^+	14	H^+	14
Na^+	9	Mg^{2+}	5	H^+	5	Mg^{2+}	3	Na^+	6	Mg^{2+}	7	Na^+	6	Na^+	4	K^+	8	Na^+	10
K^+	2	K^+	2	K^+	2	K^+	2	K^+	2	K^+	2	K^+	2	K^+	3	H^+	3	K^+	2
SO_4^{2-}	42	SO_4^{2-}	25	SO_4^{2-}	42	SO_4^{2-}	26	Cl^-	34	SO_4^{2-}	57	Cl^-	39	Cl^-	39	Cl^-	65	Cl^-	58
NO_3^-	18	NO_3^-	24	NO_3^-	23	NO_3^-	25	SO_4^{2-}	26	NO_3^-	39	SO_4^{2-}	24	NO_3^-	21	SO_4^{2-}	32	SO_4^{2-}	32
Cl^-	14	Cl^-	13	Cl^-	5	Cl^-	6	NO_3^-	15	Cl^-	28	NO_3^-	16	SO_4^{2-}	20	NO_3^-	22	NO_3^-	31

precipitation acidity suggested by Stensland and Semonin (1982) and Munger (1982) were not obvious at ELA. Comparison of our 1980 bulk and wet-only samples showed that in spite of higher concentrations with Ca^{2+}, Mg^{2+}, and NH_4^+ in the bulk samples, H^+ values were also higher. As well, no correlation, positive or negative, was found between H^+ and Ca^{2+}, Mg^{2+}, or K^+ in more than 400 individual bulk event samples, indicating that these cations were not associated with proton scavenging anions. In fact, hydrogen concentration in these samples was positively correlated with NH_4^+.

Munger (1982) illustrated decreasing H^+ deposition from east to west across northern Minnesota and North Dakota, and attributed this to "natural" buffering. Our evidence suggests that this gradient exists due to the decreasing influence of emissions originating in the lower Great Lakes region. The geographical extent of the transport of these emissions is obviously controlled by atmospheric conditions which are tremendously variable. Although the deposition of H^+ at ELA was consistently low until 1982, it appeared that the area was occasionally subjected to low pH precipitation, particularly during the winter, and dependent upon atmospheric fluctuations. Our proximity to areas of high SO_x and NO_x emissions suggests that ELA lakes and watersheds are not immune to future acidification.

Acknowledgments

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Annual Supply of ^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, ^{210}Po, and ^{232}Th to Lake 239 (Experimental Lakes Area, Ontario) from Terrestrial and Atmospheric Sources

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The activities of ^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, ^{210}Po, and ^{232}Th were measured in seasonal samples of stream runoff water, suspended and bedload sediments, and bulk precipitation. These nuclides were also determined in stream drainage area soils and bedrock. From these measurements and hydrological data, an estimate of the annual supply of these nuclides to Lake 239 at the Experimental Lakes Area (ELA) was made. Because of low concentrations of major ions, suspended sediments, and low pH, many common stream transport mechanisms for these nuclides are not available, and we propose that dissolved organic carbon is important in this respect. The annual flux (per unit stream drainage area) of these nuclides to the lake is similar to fluxes calculated for larger rivers of the world. Uranium-234 is transported preferentially to ^{238}U at activity ratios of 1–1.3. Radium-226 is carried in solution in proportion to Ca^{++}. The major supply of ^{210}Pb to the lake is from atmospheric fallout on the lake surface area. There are insufficient ^{222}Rn or ^{210}Pb deficits in the soil profiles to balance the measured excess ^{210}Pb in surface soils and lake sediments. Partition coefficients for these nuclides were calculated from data on stream suspended sediments and stream water; U and Ra coefficients were $\approx 10^4$, and Th, ^{210}Pb, and ^{210}Po coefficients were 10^4 to 10^5.

On a déterminé l'activité des nuclides ^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, ^{210}Po et ^{232}Th dans des échantillons d'eau de ruissellement, de sédiments en suspension, de sédiments charriés sur le lit et de produits de précipitation prélevés à des saisons différentes. Ces nuclides ont aussi été dosés dans le sol et la roche mère du bassin versant du cours d'eau. On a, à partir de ces valeurs et de données hydrologiques, estimé l'apport annuel de ces nuclides au lac 239 de la zone de lacs expérimentaux. Étant donné les faibles concentrations des principaux ions, la présence de sédiments en suspension et le faible pH, bon nombre des mécanismes courants du transport des nuclides par cours d'eaux sont absents, et les auteurs émettent l'hypothèse que le carbone organique dissous joue un rôle important dans le présent cas. L'apport annuel (par unité de surface du bassin versant) des nuclides est semblable aux apports calculés, au niveau mondial, pour les plus importants cours d'eau. Il y a transport préférentiel du ^{234}U par rapport au ^{238}U, à des rapports d'activité de 1–1.3. Le ^{226}Ra est transporté en solution en proportion du Ca^{++}. Le principal apport de ^{210}Pb vers le lac provient des précipitations atmosphériques tombant sur la surface du lac. Les déficits en ^{222}Rn ou ^{210} Pb des profils du sol sont insuffisants pour équilibrer le ^{210}Pb présent en excès dans les sols de surface et les sédiments lacustres. Les coefficients de répartition de ces nuclides ont été calculés à partir de données sur les sédiments en suspension et l'eau du cours d'eau; les coefficients du U et du Ra se situaient aux environs de 10^4 et ceux du Th, du ^{210}Pb et du ^{210}Po se situaient entre 10^4 et 10^5.

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Global cycles of some elements are being altered by human and technological activities (Garrels et al. 1973; National Research Council 1981; Barney 1980; Svensson and Söderlund 1976). Estimation of elemental global cycles requires measurements of seasonal and annual fluxes of the elements among the solid, liquid, and gas phases of the earth's surface at reference locations and also the necessary knowledge to extrapolate reference data over large tracts of unmonitored land and sea. For example, Ku et al. (1977) attempted a uranium mass balance of the world ocean based upon oceanic activities and world average river inflow, the latter average being derived from very few measurements on waters of the Congo, Amazon, Mississippi, and some Asian rivers. To our knowledge, little work has been done to quantify local or global mass balances of ^{230}Th, ^{226}Ra, ^{210}Po, and ^{210}Pb.

As fossil fuel supplies dwindle, the next major energy sources may be nuclear fission and fusion reactors. The largest environmental hazard of this energy source will not be spent reactor fuel, but the wastes from uranium mining and milling (Wrenn and Jinks 1976). Uranium mine wastes have caused environmental problems in downstream receiving waters (Kalin and Sharma 1982). Few data are available for seasonal and annual fluxes of uranium series elements from the land to aquatic ecosystems. Few quantitative, whole watershed data are available for comparison to disturbed areas (naturally enriched uranium-bearing watersheds, mining and milling sites).

The uranium and thorium series nuclides are useful tools in

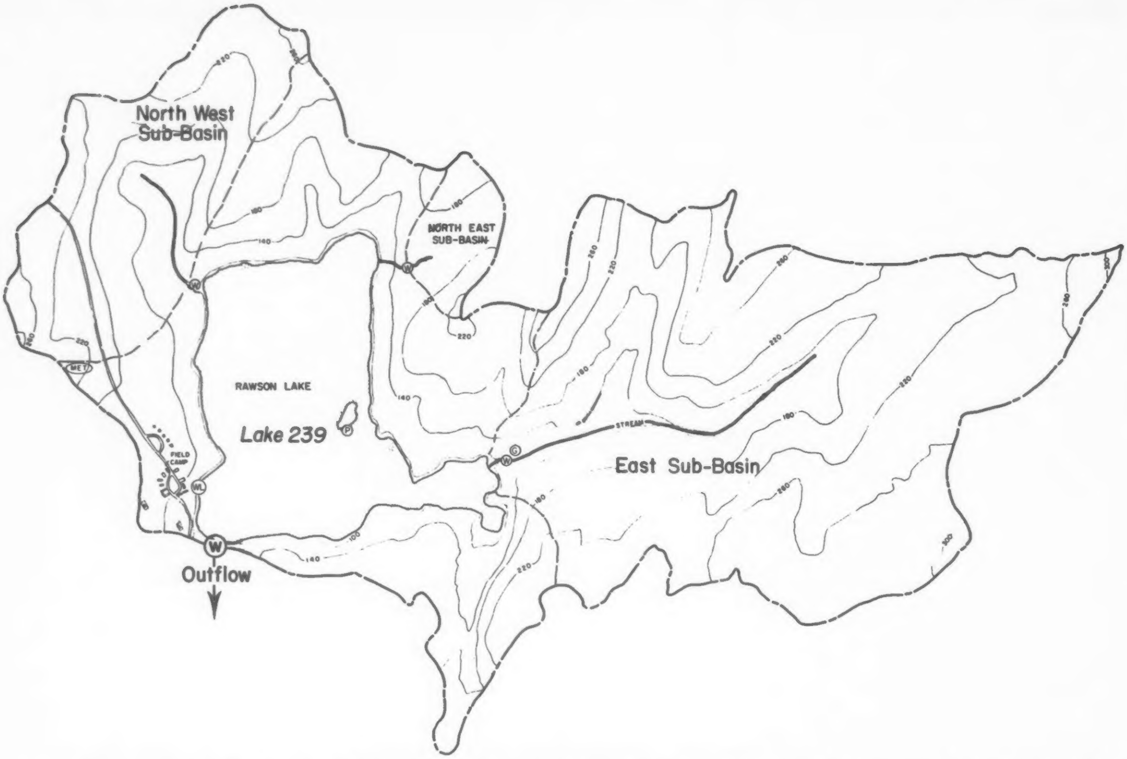

FIG. 1. Topographical map of the Lake 239 drainage basin (adapted from Beaty 1981). w = weir for monitoring water discharge, and radiochemical sampling location. MET = meteorological site of precipitation collections for ^{210}Pb measurements in rain water. The interrupted line (---) marks the boundaries of the Northwest, Northeast, and East drainage basins. The remainder of the lake drainage area is not monitored for runoff, except at the outflow weir.

ecological research, especially ^{226}Ra and ^{222}Rn (Emerson 1975) and ^{210}Pb (Robbins 1978). The disequilibrium of ^{226}Ra and ^{222}Rn has been used to estimate gas exchange rates between water and the atmosphere, and ^{210}Pb is commonly used to estimate sedimentation rates, sediment age, and the thickness of the mixed layer of sediments. Knowledge of the natural biogeochemical cycles of these elements will allow scientists to test and improve the application of these nuclide tools to other research problems. For these reasons, we want to describe the annual mass balances of these nuclides in a small lake ecosystem involving the compartments of the terrestrial drainage area, atmospheric inputs, lake water, sediment accumulation rates, and selected members of the aquatic food chain. In this paper we report on the stream drainage area and atmospheric inputs of nuclides to Lake 239 at the Experimental Lakes Area (ELA), in northwestern Ontario. There are good records of hydrological, chemical, and biological data for this region, and it is similar in climate, geology, and vegetation to the uranium mining areas of Canada, such as Beaverlodge Lake in northern Saskatchewan (Swanson 1983) and Elliott Lake in Ontario (Moffett and Tellier 1978).

Study Area

The Experimental Lakes Area (ELA) is located 52 km southeast of Kenora, Ontario, at 93°45′W and 49°40′N, in a region of boreal forest. This region has a temperate zone climate, with a 30-yr mean annual temperature of +2.1°C and mean annual precipitation of 685 mm, 31% of which is delivered as snow. Average frost-free season is from 20 May to 26 September or 128 d. The region is underlain by Precambrian granites, with irregular thicknesses of glacial drift boulders, gravels and sands in the lower areas. The area was part of glacial Lake Agassiz 8000 to 12 000 years ago, and now drains to the Winnipeg River, which contributes to the Nelson River drainage area. ELA is well described in the literature (Brunskill and Schindler 1971; Schindler et al. 1976; Newbury et al. 1979; Schindler et al. 1980; Newbury and Beaty 1980). We sampled a stream from each of the two subbasins (Northwest Basin and East Basin) draining part of the watershed of Rawson Lake (Lake 239, Fig. 1). The NE subbasin was not sampled because of its small area. These stream basins were affected by a severe windstorm in 1973, and the East Basin was burned in June 1974. Both stream basins were again partially burned in June 1980 by forest fires. Chemical and hydrological measurements have been made on these basins since 1969 (Schindler et al. 1976, 1980; Beaty 1981).

Methods

Sample Collection

Three bedrock samples from ELA Lake 239 drainage areas

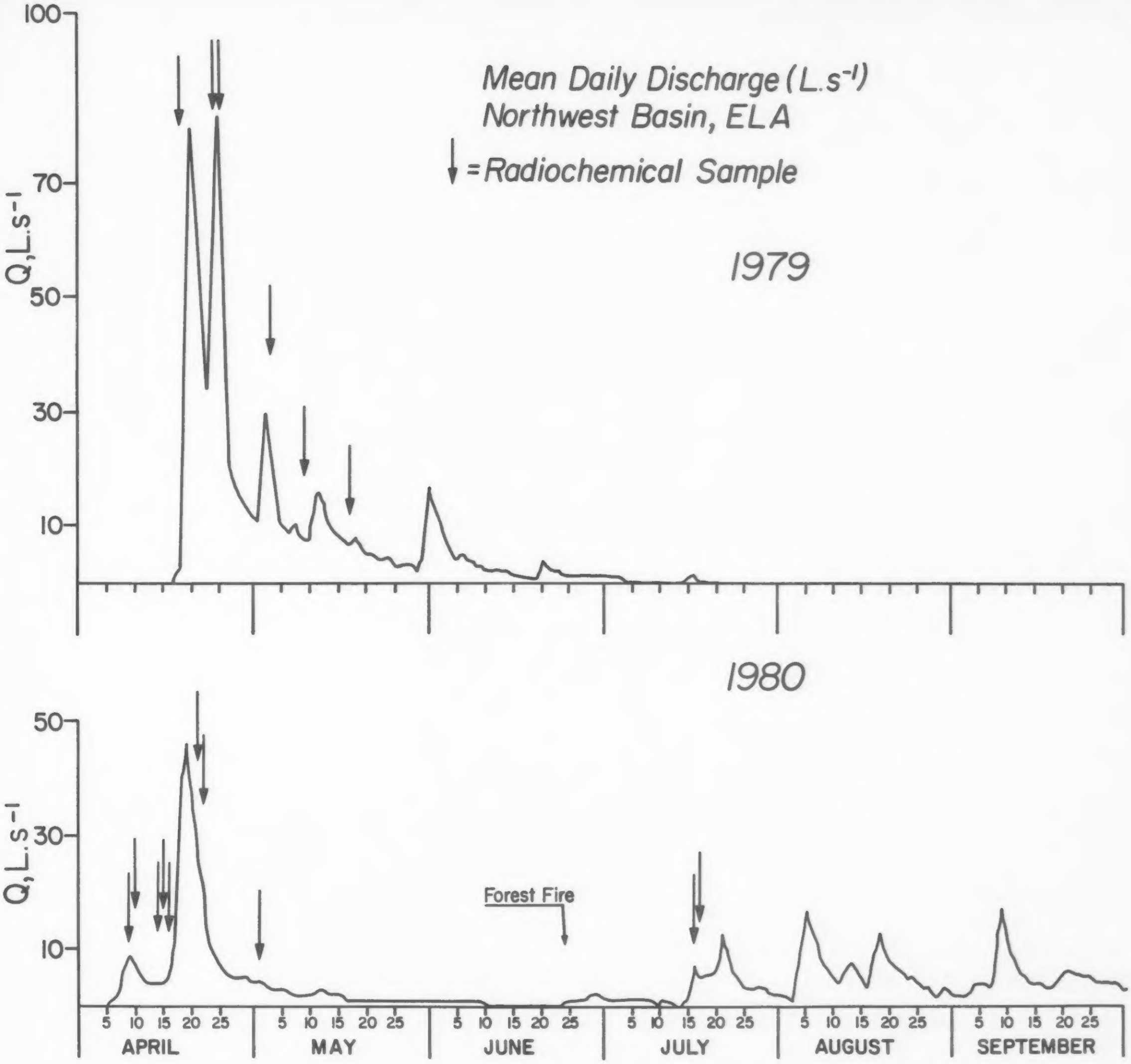

FIG. 2. Water discharge for the Northwest basin stream flowing into Lake 239 for 1979 and 1980. Data from K. Beaty, pers. comm.

were obtained by drilling into bedrock beneath overburden during installation of groundwater monitoring wells (Beaty 1981, p. 326). Our samples are from drill core sections from 60 to 80 cm below the bedrock surface at a location near the mouth of the Northwest Basin (Fig. 1). This unweathered granodiorite was similar in appearance to all other exposures of bedrock in the Lake 239 drainage area (Brunskill et al. 1971).

Three soil profiles were obtained by cutting 5 or 10 cm square sections from the side of a shovelled trench, using a knife and a stainless steel kitchen spatula. The A, B, and C horizons of the soil profile were selected in the field based upon soil color and texture. Sampling locations (NW3, EB1, EB2) were in the stream valley near the mouths of the streams where soil development occurs. Stream basin slopes and uplands have thin or no soil.

Stream bedload samples were obtained from a bedload trap installed 138 m upstream from the mouth of the East Basin stream of Lake 239 (Beaty 1981, p. 357). The bedload trap was emptied and sampled 2–3 times each year from 1974 to 1982. Bedload samples from the Northwest Basin at ELA were taken by hand from sediment accumulation just above the weir.

Suspended sediment was obtained by filtration and by centrifugation of large (50–200 L) volumes of water. Samples were filtered in the field by pumping stream water through 8 and 1.2 μm pore diameter cellulose acetate filters mounted in a 142 mm filter holder. The pump had plastic impellers, and rubber or plastic hose was used to collect, filter and direct 55–220 L samples to plastic-lined barrels. Suspended sediment chemistry data were obtained from the difference between filtered and unfiltered barrel measurements, and also by direct analyses of centrifuged suspended sediment. Cen-

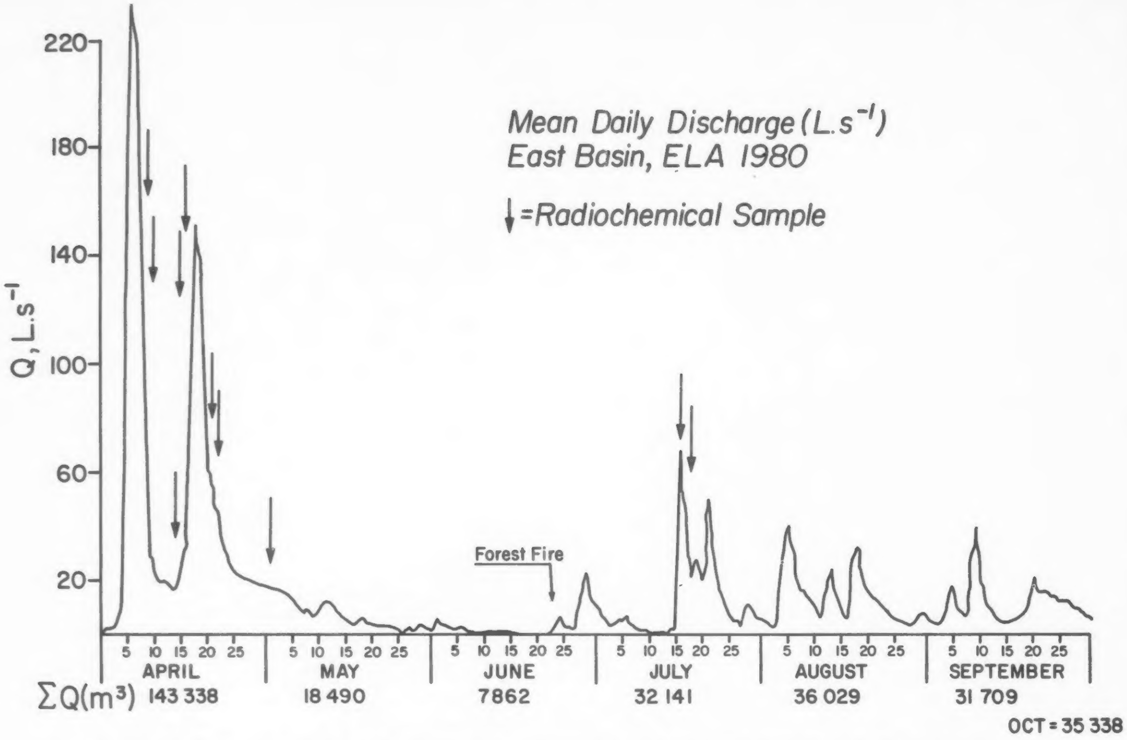

FIG. 3. Water discharge for the East Basin stream flowing into lake 239 for 1980. Data from K. Beaty, pers. comm.

trifugation of 55 L samples was done in the Freshwater Institute laboratory, months after sampling using a Sorvall KSB continuous flow system coupled to a Sorvall RC2-B centrifuge (flow rate = 40 mL·min^{-1} at 16 000 rpm). Samples of wet and dry (bulk) atmospheric precipitation were collected in 1.2 to 2.7 m^2 plastic-lined rain collectors at the ELA meteorological site. Unfiltered precipitation samples were scavenged with FeOOH as described below.

Stream runoff samples were collected at the stream weir in 60 L capacity "deldrums" (Container Corp. of America®, Willmington, Del.) and shipped to the Freshwater Institute for analysis. Water discharge from ELA research basins was monitored by automatic and manual methods (Beaty 1981). Occasional samples of suspended sediment from the East Basin stream were taken over 1978–83 (Beaty 1981 and K. H. Beaty, Department of Fisheries and Oceans, Winnipeg, Manitoba, pers. comm.). Water samples for major stable elements and nutrients were taken weekly during the runoff season (mid-April to November) by camp staff, and were analyzed according to methods given in Stainton et al. (1977). Stream water samples for radiochemical analyses were taken by ELA hydrologists during high flow periods (Fig. 2, 3). Low flow periods were sampled less frequently because they supply relatively small inputs in the mass balance.

Analytical Methods

Our radiochemical methods are described by Wilkinson (1985) and are only briefly summarized here. Filtered and unfiltered 60–200 L field samples were acidified to pH <2, spiked with yield tracers (^{232}U, ^{228}Th, ^{208}Po, and stable lead), and flocculated (with excess $FeCl_3$ addition) at pH >8. The yield tracers and ^{238}U, ^{239}U, ^{230}Th, ^{210}Pb, and ^{210}Po were extracted from the FeOOH flocc, soil and rock samples by acidification, digestion, solvent extraction, and anion-exchange columns, and the separated nuclides were plated on silver or steel discs for alpha particle counting on a silicon surface barrier detector. Radium was collected and measured by the radon emanation methods of Broecker (1965) and Mathieu (1977). All analytical tracers were calibrated to reference standards directly traceable to the National Bureau of Standards (Wilkinson 1985). Our analytical laboratory participates in both in-house and external quality assurance programs (Smith and Steger 1983). Our results are reported in units of activity, Becquerels (Bq), or disintegrations per second, and the ± error term given is either a counting error or two standard deviations of the mean value of replicate determinations. This error estimation includes uncertainties due to calibration, detector efficiencies, standard activities, and sample volume or weight.

Results

Source Materials of the Land

The common pink granodiorite bedrock exposed throughout ELA (Table 1), is rich in quartz, plagioclase, and K-feldspar, and is similar in mineralogy and chemical composition to soils and stream bedload of the Lake 239 basin. There are less common bedrock outcrops of biotite and muscovite-rich gra-

TABLE 1. Stable element composition of source materials available for weathering in the Lake 239 drainage area. See Brunskill et al. (1971) for more data on soil and bedrock geochemistry. C_0 = organic carbon. Soil horizons A, B, and C are indicated for the soil samples. % L.O.I. = loss of ignition of organic matter. Other elements are given as µmoles/g dry weight.

Sample		Ca	Mg	Na	K	Al	Si	Fe	Mn µmol/g dry wt.	CO_3-C	C_0	N	P	% L.O.I.
Lake 239 bedrock (pink granodiorite)		242	86	1 500	700	2 570	12 500	140	3.26	<17	83	<7	6.5	0.2
NW Basin soil Profile (NW3) April '81	A	287	125	996	823	2 080	10 715	211	4.12	67	2 830	79	9.7	14.7
	B	277	111	980	620	2 260	12 860	197	2.46	50	417	<7	6.5	3.8
	C	284	132	1 090	734	2 550	12 143	247	3.08	33	250	<7	6.5	0.9
E. Basin soil Profile (EB1), April '81 burned	A	304	67	692	506	1 910	11 430	174	6.78	33	1 080	36	23	9.2
	B	284	28	1 190	737	2 480	11 790	50	1.13	<17	333	<7	3.2	1.6
	C	232	14	1 150	862	2 430	12 860	38	0.96	<17	250	<7	<1	0.9
E. Basin soil Profile (EB2), (not burned) April '81	A	289	58	870	445	1 675	10 715	95	3.26	67	3 420	71	6.5	19.2
	B	302	50	1 040	620	2 290	13 570	81	1.49	17	333	<7	<1	1.8
NW Basin bedload		254	20	1 205	882	2 490	13 570	65	1.14	<17	167	<7	6.5	0.8

TABLE 2. Radiochemical composition of source materials for weathering in the Lake 239 drainage basin. See Brunskill et al. (1971) for more data on soils and bedrock chemistry. Soils were sampled in April 1980.

Samples		^{238}U	^{234}U	^{230}Th	^{226}Ra mBq·g^{-1}	^{210}Pb	^{232}Th	^{228}Th
Lake 239 bedrock (pink granodiorite)								
	Sample A	28 ± 2[a]	30 ± 2	33 ± 3	26 ± 4	34 ± 5	82 ± 6	85 ± 7
	Sample B	28 ± 2	28 ± 2	27 ± 2	—	29 ± 4	84 ± 6	100 ± 8
NW Basin soil profile (NW3, not burned)								
	horizon A	92 ± 12	115 ± 20	13 ± 3	23 ± 6	56 ± 6	26 ± 4	35 ± 6
	B	12 ± 2	12 ± 2	12 ± 2	14 ± 2	7 ± 1	26 ± 4	36 ± 5
	C	15 ± 3	14 ± 4	13 ± 2	14 ± 2	7 ± 1	36 ± 5	33 ± 4
East basin soil profile (EB1, burned area),								
	horizon A	21 ± 3	20 ± 3	12 ± 2	6 ± 2	220 ± 22	23 ± 0.9	46 ± 5
	B	15 ± 2	14 ± 1	11 ± 3	5 ± 0.9	12 ± 1	28 ± 4	49 ± 6
	C	6 ± 1	6 ± 1	6.6 ± 9	5 ± 1	4 ± 0.7	11 ± 16	28 ± 4
East basin soil profile (EB2, not burned),								
	horizon A	20 ± 3	21 ± 4	9 ± 2	11 ± 0.1	82 ± 9	15 ± 3	27 ± 6
	B	10 ± 2	10 ± 2	9 ± 2	6 ± 0.5	9 ± 1	16 ± 3	30 ± 4
East basin stream bedload, sand		13 ± 2	13 ± 2	9 ± 2	8 ± 2	9 ± 1	19 ± 4	—
East basin stream bedload, organic detritus		18 ± 3	8 ± 2	21 ± 6	12 ± 2	33 ± 17	49 ± 12	—
Suspended sediment from East Basin (A and B in Table 10)	A	60 ± 4	75 ± 5	14 ± 1	47 ± 5	216 ± 18	18 ± 2	—
	B	69 ± 4	80 ± 6	16 ± 1	32 ± 3	373 ± 29	25 ± 2	

[a] Error given is one standard deviation of the mean activity of replicate (3) subsample determinations, or average counting error, whichever is larger. A dash (—) indicates that the nuclide was not measured.

nite, quartz-veined granite, and a detrital chloritic greenstone. The sands and gravels overlying this bedrock were transported and deposited by glacial ice and sorted by glacial melt-waters and glacial Lake Agassiz (Brunskill et al. 1971). In areas where there are no glacial deposits, the bedrock is often bare, or supports lichen growth and/or 2–10 cm thick moss beds and vascular plants with very little soil development.

The weathering of the bedrock into soils and bedload material has little effect upon the major element composition in most cases (Table 1). Calcium, Na, Al, Si, and P do not vary greatly within soil profiles, especially when corrected for dilution by organic matter. Organic matter, organic carbon, total nitrogen, and Mn are enriched in soil A horizons, and Fe and Mg are depleted in some soil B and C horizons. These sandy soil samples represent stream valley sediments which occur in a small area from the stream mouth and upstream 50–100 m.

TABLE 3. ^{210}Pb in wet and dry precipitation collected at the meteorological site in the Lake 239 drainage basin in 1982.

Date	Water volume collected (m^3)	^{210}Pb Activity (Bq·m^{-3})
8–16 June	0.033	530
24–30 June	0.086	2 540
1– 7 July	0.116	75
15–29 July	0.129	46
13–26 Aug.	0.029	55
9–23 Sept.	0.045	83
7–24 Oct.	0.046	34
25 Oct.–7 Nov.	0.012	427
	0.416 m^3 = 416 mm	
	1983 rain = 561 mm	
	1983 snow = 147 mm	

TABLE 4. Atmospheric flux of ^{210}Pb to ELA soils and lake surfaces from precipitation collections, and calculated fluxes required to maintain excess ^{210}Pb integrals in Lake 239 drainage area soils and lake sediments (Kipphut, pers. comm.).

Source	Flux of ^{210}Pb (excess) Bq·m^{-2}·yr^{-1}
Wet and dry precipitation (from Table 3)	77
Lake 239 drainage area soils (from Table 2)	26–111
Lake 239 sediment cores (Kipphut, Institute of Marine Science, Fairbanks, Alaska, pers. comm.)	126–344

TABLE 5. Characteristics of the drainage basin of Lake 239, ELA. Data are from hydrological summaries by Beaty (1981 and personal communication). Lake 239 surface area is 0.543 km^2.

Region	Area (km^2)	Annual discharge (10^3 m^3)	Runoff (cm)
East Basin	1.703		
1979		248	15
1980		273	16
1970–82 Mean		367	22
Northwest Basin	0.5638		
1979		76.2	14
1980		80.7	14
1970–82 Mean		103.7	18
Total lake drainage	3.905		
1979		735	19
1980		491	13
1970–82 Mean		961	25

TABLE 6. Annual supply of suspended and bedload sediments to Lake 239 from the East Basin drainage area (K. Beaty, 1981 and personal communication).

	Dry weight (tonnes·yr^{-1})	Drainage area flux (g·m^{-2}·yr^{-1})
Suspended sediment (1978–83)	0.58 to 2.0	0.34 to 1.2
Bedload[a] (1976–82)	0.8 to 8.8	0.47 to 5.2
Bedload[a] (1979–80)	0.8 to 1.3	0.47 to 0.7

[a] Bedload here is the largely inorganic material collected in a bedload sediment trap installed in the streambed. Large quantities of floating organic debris are not collected by this method. See Beaty (1981 Part III, p. 356).

The remainder of the basin is bare rock, moss-covered rock, and thin organic soils.

The granodioritic bedrock sample results (Table 2) show radioactive equilibrium for the daughters of ^{238}U (^{234}U, ^{230}Th, ^{226}Ra, and ^{210}Pb) and ^{232}Th (^{228}Th). Uranium activities are higher in suspended sediments and the A horizons of the NW and East Basin soil profiles, and are present at lower activities in the B and C horizons of soil and in the stream bedload samples. There is a large deficiency of ^{234}U (relative to ^{238}U) in the organic matter (leaves, woody debris) of stream bedload from the East Basin. Uranium activities are equal to or greater than daughter ^{230}Th in these soil and bedload samples, which suggests little preferential weathering of U compared to Th, or that U is preferentially sorbed to these particles. Stream suspended sediments are also enriched in U (Table 2).

Thorium isotope activities are more uniform in the soil, bedload, and suspended sediment (Table 2). Thorium is not greatly partitioned between the soil horizons, with the exception of the anomalous C horizon of the East Basin burned soil profile. In general, there is an excess of ^{228}Th in the soil samples, compared to its grandparent ^{232}Th.

Radium-226 is enriched in organic-rich A horizons of some ELA soils. Radium activities for most soil samples are near equilibrium with the parent ^{230}Th, suggesting little preferential weathering of ^{226}Ra. However, suspended sediments carry 2 times more ^{226}Ra than its parent ^{230}Th. Lead-210 is greatly enriched in the surface soils because of input from atmospheric ^{210}Pb (see below). Deficiencies of ^{210}Pb (relative to grandparent ^{226}Ra) in the B and C horizons of soil profiles are likely the result of loss of ^{222}Rn to the atmosphere.

Atmospheric Source of ^{210}Pb

Lead-210 in wet plus dry precipitation for the Lake 239 drainage area (Table 3) varied from 30 to 2500 Bq·m^{-3}. These ^{210}Pb activities were converted to flux (Bq·m^{-2}·yr^{-1}) data by summing ^{210}Pb activities and the volume of water collected to estimate average ^{210}Pb activity per m^{-3} of rain water, and then multiplying this average by total water precipitation for the year. The resulting annual flux of about 80 Bq·m^{-2}·yr^{-1} (0.25 atoms·cm^{-2}·yr^{-1}) is in the middle of the range of values for calculated ^{210}Pb fluxes to Lake 239 drainage area soils (Table 4). Atmospheric excess ^{210}Pb fluxes to the surface layers of soil profiles (Table 2) were obtained by dividing the unit area integral of excess ^{210}Pb in the soil (total ^{210}Pb–^{226}Ra) by the mean life of ^{210}Pb (32 yr). The calculation yielded the average annual atmospheric ^{210}Pb flux necessary to maintain the observed excess of ^{210}Pb in the surface soil samples.

Bedrock, soils, and atmospheric deposition are the sources of nuclides for transport into Lake 239. We will now consider the annual transport rates of these nuclides carried to the lake by stream water, suspended and bedload sediments, and precipitation.

TABLE 7. Stable element concentrations in Lake 239 drainage area streams, 1980, during high and low discharge. DIC = dissolved inorganic carbon, DOC = dissolved organic carbon. dSi = soluble reactive silicon.

Date	Discharge m^3·d^{-1}	Specific Conductance µmhos·cm^{-1}	pH	Ca	Mg	Na	K	Cl	SO$_4$	DIC	DOC	dSi
				µmol·L^{-1}								
NW Basin												
23 April	1 123	21	5.15	39	15	36	3.1	28	50	182	1 160	113
24 July	432	71	5.15	117	77	83	19	90	231	324	945	—
East Basin												
23 April	2 940	26	6.00	64	23	56	12	28	54	170	1 340	139
10 July	86	69	6.40	146	79	123	32	59	136	504	1 285	245

TABLE 8. Uranium series nuclides in stream runoff water samples from the Northwest drainage area of Lake 239, Experimental Lakes Area, northwestern Ontario. ND = not detected; UF = unfiltered; F = filtered; Cent = centrifuged.

Date	Treatment	Discharge[d] (m^3·d^{-1})	Suspended sediment (g·m^{-3})	^{238}U	^{234}U	^{230}Th	^{226}Ra	^{210}Pb	^{210}Po	^{232}Th
							Bq·m^{-3}			
17 Apr. 1979	UF	86	—	1.1 ± 0.1[c]	2.8±0.2	0.34±0.05	1.8±0.1	—	—	0.36±0.05
24 Apr.	UF	4 147	—	7.5±12	34 ±6	ND	0.93±0.29	—	—	ND
25 Apr.	UF	7 171	—	8.0±0.8	7.9±0.8	1.0±0.1	1.6±0.1	—	—	1.1 ±0.10
3 May	UF	1 987	—	39 ±7	15 ±2.8	ND	1.2±0.3	—	—	0.41±0.05
9 May	UF	691	—	11 ±1.6	8.1±1.3	ND	0.85±0.11	—	—	ND
17 May	UF	605	—	4.8±0.6	2.3±0.4	ND	0.96±0.15	—	—	0.41±0.05
9 Apr. 1980	Cent	788	1.0	3.7±0.6	4.3±0.7	0.16±0.002	0.93±0.37	—	—	0.25±0.03
10 Apr.	Cent	605	0.9	2.3±0.2	1.3±0.1	0.24±0.03	0.74±0.26	—	—	0.26±0.03
14 Apr.	Cent	346	1.6[d]	5.0±0.8	6.0±1	0.12±0.02	0.81±0.19	4.3±0.5	2.5±0.2	0.19±0.03
15 Apr.	Cent	346	—	5.6±0.5	6.0±0.5	ND	0.74±0.26	4.1±0.5	13 ±1.9	ND
16 Apr.	UF	432	—	4.3±0.5	4.5±0.5	0.17±0.01	1.07±0.52	2.6±0.3	15 ±1.6	0.27±0.02
21 Apr.	Cent	2 333	2.0	—	—	—	0.93±0.22[a] 0.67±0.15[b]			
22 Apr.	Cent	1 901	3.2[d]	2.1±0.6	2.9±0.8	0.21±0.04	1.4±0.2[a] 0.63±0.03[b]	—	—	0.35±0.06
1 May	Cent	346		4.9±0.5	5.4±0.5	0.18±0.03	1.7±0.2	1.9±0.2	2±0.3	0.30±0.04
16 July	Cent	605	0.6	9.1±1.2	10.6±1.4	0.26±0.03	2.4±0.3[a] 2.8±0.5[b]	5.5±0.6	9.3±2.0	0.37±0.03
17 July	Cent	432	1.4	7.9±0.7	6.6±0.9	0.14±0.01	1.9±0.1[a] 1.9±0.1[b]	4.0±0.5	4.2±0.4	0.20±0.01
10 Apr. 1981	F	490	—	1.5±0.1	1.7±0.1	0.14±0.01	1.9±0.2	1.2±0.1	1.5±0.1	0.21±0.02

[a] Before centrifugation.

[b] After centrifugation.

[c] ±2 standard deviation of measureable sources of error (counting, tracer activities).

[d] Data from K. Beaty, ELA hydrologist.

Hydrology and Sediment Transport as Nuclide Carriers

Annual runoff from the entire Lake 239 basin outflow (Fig. 1) varied from 13 to 25 cm (over 1970–82), and the period of study reported here (1979 and 80) represents below average waterflow years (Table 5). Variation in hydrological years is probably the major factor controlling variations in stable element and radionuclide annual fluxes from drainage areas (Schindler et al. 1976). There was usually a snowmelt peak runoff in April, with rainstorm peaks in July–September (Fig. 2 and 3). These drainage basins are usually frozen from December to March.

Table 6 indicates the small annual transport of suspended and bedload sediments from the drainage area of the East Basin stream. Probably as a result of the 1974 fire in the East Basin (Schindler et al. 1980), bedload transport was high (1.5–4 m^3·yr^{-1}) in 1976–77 (when the bedload measurements began). Bedload transport decreased to <1 m^3·yr^{-1} from 1978 to 1982, with the 1980 forest fire having little effect on this parameter.

Suspended sediment concentrations were usually in the range of <1 to 10 g·m^{-3} (Tables 8, 9, and 10); higher concentrations (30 g·m^{-3}) occurred during a few hours of the ascending limb of rainstorm hydrographs (K. Beaty, pers. comm.). The East Basin stream channel is well developed and drops 20 m in its 900 m length. Water residence time should be short in the stream channel. Suspended sediment transport rates for the East Basin are lower than bedload transport rates (Table 6). Suspended and bedload sediment transport has not been measured in the NW Basin, but we expect that sediment transport there is considerably lower than in the East Basin because of its thinner and more coarsely grained soils, smaller discharge, and lack of stream channel development. Runoff from the Northwest Basin is damped and filtered by percolation through an area of bog.

TABLE 9. Uranium series nuclides in stream runoff water samples from the East drainage area of Lake 239, Experimental Lakes Area, northwestern Ontario. ND = not detected; Cent = centrifuged: UF = unfiltered, F = filtered.

Date	Treatment	Discharge[d] ($m^3 \cdot d^{-1}$)	Suspended sediment ($g \cdot m^{-3}$)	^{238}U	^{234}U	^{230}Th	^{226}Ra $Bq \cdot m^{-3}$	^{210}Pb	^{210}Po	^{232}Th
9 Apr. 1980	Cent	5 962	1.3	1.6±0.1	2.2±0.2	0.15±0.02	0.70±0.07	3.0±0.3	39.0±1.5	0.23±0.03
10 Apr.	Cent	1 987	1.1	7.8±0.6	4.8±0.4	0.14±0.01	0.96±0.07	3.1±0.4	11 ±1.6	0.21±0.02
14 Apr.	UF	1 469	1.9[d]	3.3±0.5	2.9±0.5	0.15±0.01	0.63±0.05	3.5±0.4	8.0±1.6	0.23±0.01
15 Apr.	Cent	2 074	5	2.8±0.2	4.0±0.3	0.11±0.01	1.1 ±0.1[a] 0.67±0.22[b]	6.0±0.7	2.1±0.2	0.16±0.01
16 Apr.	UF	3 024	2 [d]	3.1±0.3	4.0±0.3	0.21±0.02	1.2 ±0.30	3.1±0.4	56 ±4.3	0.28±0.02
21 Apr.	Cent	4 320	9	2.6±0.3	2.9±0.3	0.19±0.02	1.2 ±0.0[a] 0.44±0.02[b]	12 ±1.5	9.9±1	0.23±0.02
22 Apr.	Cent	3 888	3.5[d]	2.0±0.2	2.8±0.3	0.16±0.02	0.89±0.03[a] 0.63±0.11[b]	0.44±0.07	—	0.36±0.03
1 May	Cent	1 555	1.4	2.4±0.2	3.4±0.3	0.16±0.01	0.48±0.11[a] 0.52±0.11[b]	1.9±0.2	1.4±0.2	0.24±0.02
16 July (0910)	Cent	5 960	30	2.9±0.3	3.8±0.4	0.30±0.03	3.6 ±0.3[a] 2.2 ±0.04[b]	1.8±0.2	1.9±0.2	0.35±0.07
16 July (1505)	Cent		10	2.4±0.2	3.6±0.3	0.18±0.01	2.4 ±0.7[a]	1.1±0.1	7.1±0.7	0.24±0.02
17 July	Cent	3 195	3.1	2.1±0.1	3.0±0.3	0.18±0.01	1.9 ±0.1[a] 1.8 ±0.1[b]	0.94±0.16	0.84±0.3	0.27±0.02
9 Apr. 1981	UF	1 253	—	2.3±0.2	3.5±0.3	0.25±0.02	0.93±0.17	—	—	0.39±0.03
9 Apr.	F			1.7±0.1	2.6±0.2	0.16±0.01	0.98±0.25	3.7±0.4	2.8±0.3	0.26±0.02

[a] Before centrifugation.

[b] After centrifugation.

[c] ± standard deviation of measureable sources or error (counting, tracer activities).

[d] Data from K. Beaty, ELA hydrologist.

Nuclides in the Solution Phase of Runoff

Stable element concentrations (from weekly sampling, April to November) are available for these two streams (Schindler et al. 1976, and unpublished data for 1976–83). Table 7 gives examples of the very low concentrations of major elements during high and low flow periods. Inorganic major elements vary in concentration by a factor of 2 to 5, but dissolved organic carbon varies by a factor of <2. The uranium-series nuclides are thought to be transported in solution as complexes or ligands with major ions or organic matter. These data were used below to compute annual fluxes of these elements from the drainage area (see below).

The transport of nuclides to the lake by streamflow depends upon the rate of leaching of soil particles, suspended and bedload sediment transport, water discharge, and atmospheric outputs. Activities of ^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb, ^{210}Po, and ^{232}Th in filtered, unfiltered, and centrifuged stream water samples are given in Tables 8 and 9, along with water discharge and suspended sediment concentrations. The 1979 data for the NW Basin are from unfiltered samples, and the high U activities may have resulted from higher concentrations of suspended sediments. Much less variation in nuclide activity is observed in the 1980 data for this basin, based upon centrifuged water samples. Uranium-238, ^{234}U, ^{226}Ra, ^{210}Pb, and ^{210}Po are carried in filtered water at activities of $1-10\ Bq \cdot m^{-3}$, whereas ^{230,232}Th activities are an order of magnitude lower (Table 8). Lead 210 activities are a factor of 2 to 5 higher than grandparent ^{226}Ra, and ^{210}Po varies greatly from 1 to 6 times the activity of its parent ^{210}Pb in NW Basin runoff water. The centrifuged water samples from the East Basin stream in 1980 (Table 9) show little variation in activities of U and Th, but ^{226}Ra, ^{210}Pb, and ^{210}Po vary by factors of 5 to 50. Many of these samples indicated that ^{226}Ra, ^{210}Pb, and ^{210}Po are considerably in excess of their parent activities. It is difficult to perceive any relationship between these nuclide activities and water discharge for the NW and East Basin streams.

Particulate Phase of Runoff

As best we can determine, much of the U and Ra is carried "in solution" (i.e. is not separated from the solution by centrifugation or filtration), and more of the Th, ^{210}Pb, and ^{210}Po is carried in particulate form (Table 10). Three methods were used in the data in Table 10: (a) centrifugation and separate analyses of water and particulate matter, (b) separate analyses of filtered and unfiltered water samples, and (c) analyses of filtered water and the filter paper. Methods (a) and (c) are obviously preferred, as they allow a direct determination of the particulate phase activity. Accumulated errors of replication and measurement are 10–20% of the given nuclide activity which means that we cannot detect particulate activities of <20% of total activities by method (b). We prefer to use the data from method a) as being most representative (Nriagu et al. 1981). The general pattern of the results of all three methods on the three different samples is similar, however, and the calculated partition coefficients for the two samples with 10 and 30 $g \cdot m^{-3}$ suspended sediments agree within a factor of three (Table 10). We should expect that 0–40% of the total sample activity for ^{238}U, ^{234}U, and ^{226}Ra will be carried in the particulate phase, and that 30–80% of the ^{230}Th, ^{232}Th, ^{210}Pb, and ^{210}Po will be carried on particles in East Basin runoff. The NW Basin results in Table 10 suggest that a larger fraction of all nuclide activities are carried in the "solution phase". Additional data for partitioning of ^{226}Ra between dissolved and particulate phases are given in Table 11, based upon the difference in ^{226}Ra activity between uncentrifuged and centrifuged samples. The calculated ^{226}Ra partition coefficient

TABLE 10. Suspended sediment: water partitioning of uranium-series nuclides in ELA Lake 239 inflowing streams. Partition coefficient = Bq·g^{-1}dry suspended sediment ÷ Bq·g^{-1} filtered or centrifuged water.

Basin	Date	Method		^{238}U	^{234}U	^{230}Th	^{226}Ra	^{210}Pb	^{210}Po	^{232}Th
East	16 July 1980 (rain storm runoff)	Centrifugation, suspended sediment analyzed directly, water analyzed separately	Sample A, 0910 hrs 30 g·m^{-3} suspended sediment							
			Total activity (Bq·m^{-3})	4.7±0.4	6.1±0.6	0.70±0.07	3.6±0.3	8.3±0.8	5.1±0.4	0.89±0.09
			% in particulate	38	37	58	39	78	63	60
			Partition coefficient	20 300 ±2 600	19 000 ±2 400	43 000 ±5 700	21 000 ±5 700	118 400 ±11 000	55 800 ±5 000	51 000 ±8 700
			Sample B, 1505 hrs. 9.9 g·m^{-3} suspended sediment							
			Total activity (Bq·m^{-3})	3.1±0.3	4.4±0.4	0.34±0.03	2.4±0.7	4.8±0.4	13.4±1.0	0.48±0.03
			% in particulate	22	18	48	13	77	47	51
			Partition coefficient	28 000 ±2 900	22 000 ±2 300	95 000 ±10 000	13 000 ±1 500	335 000 ±30 000	89 300 ±9 000	106 000 ±11 000
East	9–10 Apr. 1981	Field filtration with 8 and 1.2 μm pore size 147 mm filters, particulate activity determined from difference between unfiltered and filtered 200 L water samples (suspended sediment concentration was low)	Total activity (Bq·m^{-3})	2.3±0.2	3.5±0.3	0.25±0.02	0.93±0.15	—	—	0.39±0.03
			% in particulate	29	25	36	0	—	—	31
Northwest	9–10 Apr. 1981	Field filtration, as above	Total activity (Bq·m^{-3})	2.7±0.3	3.0±0.4	0.13±0.15	2.2±0.2	1.3±0.2	1.9±0.2	0.21±0.02
			% in particulate	44	43	0	14	8	21	0
		Analysis of filters from the above field filtration	% in particulate	7	7	13	0.2	18	—	10

varies by an order of magnitude in the East Basin, and the higher partition coefficients (during spring runoff) are much greater than those shown in Table 10.

The activities of U, Ra, and ^{210}Pb in suspended sediments of these streams are higher than in soil and stream bedload samples (Tables 2 and 11). The quantity and composition of suspended sediments varied over the seasons, but we have little information on this. Particulate carbon varies from 10 to 200 μmoles·m^{-3} in these streams over 1979 and 1980. The nuclide fluxes carried by stream bedload (Tables 2 and 6) are probably strongly controlled by discharge and drainage area disturbance (fire, drought); large bedload movements will occur during extreme high discharge and after forest fires at moderate discharge rates (Beatty 1981). We do not have annual transport data for coarse particulate organic matter (Table 2).

Discussion

We have now assembled most of the data necessary to compute the annual input of uranium-series nuclides to Lake 239, from bedrock, soils, atmospheric fallout, stream flow solution and suspended sediments, stream bedload, and water discharge. We estimate the atmospheric ^{210}Pb flux to the lake surface area and terrestrial drainage area to be around 80 Bq·m^{-2}·yr^{-1}, based upon measured rain and soil data in Tables 3 and 4. This value is in the lower third of the range of values summarized by Turekian et al. (1977), Robbins (1978), Nozaki et al. (1978) and Ophel et al. (1983). Most of the activities of ^{210}Pb in rain (plus dry fallout) samples were in the range of values reported by Ter Haar et al. (1967) for Argonne, Illinois precipitation. We are continuing our sampling of rain in order to determine the variation in annual flux of atmospheric ^{210}Pb to this region. We have recently analyzed a few precipitation samples for U, Th, and Ra, and their activities are very low, contributing little to the mass balance.

Table 12 gives our estimate of the annual transport rate of these nuclides from the rocks and soils of the two larger drainage basins flowing into Lake 239. The Northwest drainage basin is smaller and has thinner soils, and a larger proportion of exposed bedrock surface area (Beaty 1981, Part III). Suspended sediment concentrations are low even during high discharge events (Tables 8 and 11), and it appears that all nuclides are carried primarily in the "solution" phase. Based upon 1970–85 observations of the small accumulation of bedload materials behind the Northwest stream V-notch weir, we assume that bedload transport of nuclides is unimportant.

The East Basin weathering rates for these nuclides (Table 12) indicate a more important transport of Th, ^{210}Pb, and ^{210}Po in the particulate phase. The East Basin is larger, has thicker and finer-grained soil horizons in the low areas (Beaty 1981); it also carries larger suspended and bedload sediment concentrations (Tables 6, 8, and 9). Bedload transport in the East Basin is an important fraction of Th input, but contributes only a small fraction of the total input of the other nuclides. The differences between the East and Northwest drainage basins for Th, ^{210}Pb, and ^{210}Po transport rates are largely caused by the addition of a larger suspended sediment and bedload flux in the

TABLE 11. Distribution of ^{226}Ra between solution and particulate phases in runoff samples from the East and Northwest drainage basins of Lake 239, Experimental Lakes Area, northwestern Ontario. Particulate ^{226}Ra data were derived from the difference between before and after FWI laboratory centrifugation of a 55 L sample. Errors given are two standard deviations of three determinations on the same barrel.

Date	Suspended sediment g·m^{-3}	Solution ^{226}Ra Bq·m^{-3}	Particulate ^{226}Ra m Bq·g^{-1}	Partition coefficient
East drainage basin, Lake 239, ELA				
15 Apr. 1980	5.0	0.70 ± 0.22	85 ± 52	120 000 ± 110 000
21 Apr.	8.6	0.44 ± 0.03	93 ± 15	211 000 ± 37 000
22 Apr.	3.5	0.63 ± 0.11	78 ± 33	124 000 ± 72 000
1 May	1.4	0.52 ± 0.11	0[a]	
16 July (0910)	30	2.2 ± 0.03	44 ± 11	20 000 ± 5 700
16 July (1505)	9.9	2.1 ± 0.1	28 ± 74	13 000 ± 35 000
17 July	3.1	1.8 ± 0.1	0[a]	
Northwest drainage basin, Lake 239, ELA				
21 Apr. 1980	2	0.67 ± 0.15	144 ± 133	210 000 ± 230 000
22 Apr.	3.2	0.63 ± 0.03	233 ± 81	370 000 ± 140 000
16 July	0.6	2.8 ± 0.5	0[a]	
17 July	1.4	2.0 ± 0.1	0[a]	

[a] Zero particulate radium means that there was no significant difference between the centrifuged and uncentrifuged sample determinations.

TABLE 12. Annual flux (per unit drainage area) of uranium-series nuclides from ELA Lake 239 inflowing streams. SS = suspended sediment.

Basin	Year	Fraction	^{238}U	^{234}U	^{230}Th	^{226}Ra Bq·m^{-2}·yr^{-1}	^{210}Pb	^{210}Po	^{232}Th
Northwest	1979	Dissolved plus SS flux	3.4	1.7	0.032	0.17	—	—	0.054
	1980	"Dissolved" flux	0.89	1.1	0.024	0.23	0.53	1.5	0.034
		Calculated SS flux from Table 10 and 11	0.22	0.28	0.004	0.041	0.13	0.38	0.006
		Dissolved and SS flux	1.11	1.38	0.028	0.27	0.66	1.9	0.040
East	1980	"Dissolved" flux	0.41	0.53	0.031	0.22	0.59	3.0	0.046
		Calculated SS flux	0.10	0.13	0.062	0.055	2.4	4.5	0.069
		Bedload flux	0.019	0.019	0.013	0.012	0.013	—	0.028
		Total stream flux	0.53	0.68	0.11	0.29	3.0	7.5	0.14

East Basin. These data are for below average waterflow years (Table 5), and we expect that nuclide transport rates would increase for high discharge years. Annual suspended sediment transport of nuclides was estimated by extrapolating the data in Tables 10 and 11 to long-term suspended sediment data of Beaty (1981, pers. comm.) which is summarized in Table 6.

These annual flux data were calculated in two ways: (1) nuclide activities for a given sample (Tables 8 and 9) were multiplied by water discharge during that time interval (Fig. 2 and 3), and these weekly or monthly fluxes were summed for the discharge year and divided by drainage area, and (2) average nuclide activities from the year's samples (Tables 8 and 9) were multiplied by annual stream discharge (Table 5). The two methods gave similar results (± 10%). This indicates that variations in annual flux of these nuclides will be controlled by variations in annual discharge of water. The suspended and bedload flux is based upon fewer samples (Tables 1, 6, 10, and 11) and is the most uncertain of our input parameters. Bedload flux was calculated as the product of the 1979–80 bedload mass transport (Table 6) and the nuclide activity of triplicate samples of this sandy sediment (Table 2). The nuclide transport rates for the NW Basin will be extrapolated to all the rest of the Lake 239 drainage basin, excluding the East drainage basin area. This is because the unsampled drainage area (Fig. 1) most resembles the NW Basin characteristics (Newbury and Beaty 1980).

There are two additional sources of ^{210}Pb to the lake that are not included in Table 12. Firstly, atmospheric inputs of ^{210}Pb to the lake surface area are about 80 Bq·m^{-2}·yr^{-1} (Table 4), and secondly ^{210}Pb is added to the lake from the decay of stream water ^{222}Rn. Radon-222 activities are very high in ELA groundwater (18–65 kBq·m^{-3}, Kennedy 1974) and stream runoff. We have only one measurement in NW Basin snowmelt (10 April 1980), which gave an activity of 330 ± 250 Bq ^{222}Rn·m^{-3}. The sample was taken near the mouth of the stream, and some of the ^{222}Rn may have degassed to the atmosphere. From experience with ^{226}Ra and ^{222}Rn in ELA lakes (Emerson and Hesslein 1973; Emerson 1975), we esti-

TABLE 13. A comparison of nuclide fluxes from terrestrial drainage areas of rivers and streams, in $Bq \cdot m^{-2}$ drainage area per year.

River	^{238}U	^{234}U	^{230}Th $Bq \cdot m^{-2} \cdot yr^{-1}$	^{226}Ra	^{232}Th	Comments, reference
Lake 239 streams, ELA, Ontario	0.82	1.0	0.07	0.28	0.09	Precambian Shield granite, Boreal forest. This work.
St. Lawrence Ontario/Quebec	0.082	—	—	0.34	—	Mostly Precambrian granites Rona and Urry (1952) Krishnaswami and Lal (1978)
Ottawa, Ontario	0.19	—	—	3.4	—	Merrit (1975), Havlik (1970)
Mackenzie, NWT	2.3	—	—	—	—	Turekian and Chan (1971)
Liard, NWT	4.3	—	—	—	—	Turekian and Chan (1971) Largely sedimentary basin
Mississippi, USA	2.2	2.8	0.58	0.20[a]	0.44	Moore (1967), sedimentary basin
(% particulate)	11%	8%	94%	—	93%	
Amazon, Brazil	0.99	1.0	0.64	0.29[a]	0.92	Moore (1967)
(% particulate)	54%	50%	59%	—	62%	
Salgado, Brazil	0.60	0.88	—	—	—	Moreira-Nordemann (1984), saline water, granitic gneiss
Congo, Africa	0.48	—	—	—	—	Bertine et al. (1970)
Ganges, India	19	—	—	—	—	Bhat and Krishnaswami (1969)
Narbada, India	2.7	3.8	—	—	—	Borole et al. (1982), basaltic terrain
Tapti, India	0.8	1.0	—	—	—	" " " "

[a] Dissolved ^{226}Ra only, does not include particulate ^{226}Ra.

mate that perhaps one half of the added stream ^{222}Rn would decay to ^{210}Pb in the epilimnion of Lake 239. If we multiply 1/2 of the above ^{222}Rn activity by the 1980 discharge of the NW Basin (Table 5), and divide by the lake area and the mean life of ^{210}Pb, we obtain a ^{210}Pb input flux to the lake area of ≈ 8 $Bq \cdot m^{-2} \cdot yr^{-1}$, which is about 9% of the measured atmospheric ^{210}Pb flux (Table 4).

Most of the nuclide transport data for large river U and Ra are within a factor of three of our headwater stream ELA data, but the larger rivers carry more Th (Table 13). This is likely due to higher concentrations of suspended sediments in rivers draining sedimentary basins, such as the Mississippi (Moore 1967). Moore (1967), Spalding and Sackett (1972), Sackett et al. (1973) and Mangini et al. (1979) were concerned that U in commercial fertilizer was enhancing U activities in runoff from agricultural drainage basins. The U weathering rates for undisturbed basins of ELA streams, the subarctic Mackenzie and Liard rivers, and the tropical Amazon display a range of values equal to or greater than those of the agricultural basins (Table 13). Although U is preferentially sorbed to the organic matter of surface soils in our small basins, there is clearly a net loss of U to streamflow, in contrast to the net retention of U in organic deposits described by Levinson et al. (1984).

Field measurements (Moore 1967; Borole et al. 1982; Moreira-Nordemann 1984), experimental studies (Fleischer and Raabe 1978; Fleischer 1980a, 1982, 1983; Kobashi and Tominaga 1982) and oceanographic models (Ku et al. 1977) have documented a preferential aqueous transport of ^{234}U relative to ^{238}U in runoff. The activity ratio $^{234}U/^{238}U$ in Table 13 varies from 1.1 to 1.4 for major rivers of the world. The averages of $^{234}U/^{238}U$ for individual measurements of ELA runoff (Tables 8 and 9) are $1.24^{+0.37}_{-0.29}$ (1980 East Basin), $1.08^{+0.29}_{-0.23}$ (1980 NW Basin) and $0.73^{+0.75}_{-0.36}$ (1979 NW Basin), but the ranges of the activity ratios were 0.4 to 2.6. Unusual $^{234}U/^{238}U$ have been reported (Szabo 1982; Shirvington 1983) for water and sediments, and it was proposed that α-recoil ejection ^{234}U is preferentially sorbed to mica-illite (Shirvington 1983), which is a common clay mineral in ELA soils and lake sediments (Brunskill et al. 1971). The bedrock granite $^{234}U/^{238}U$ is near 1.0, whereas stream suspended sediment activity ratios (1.1−1.25) generally show a small enrichment of ^{234}U (Tables 2 and 10). This requires that there be a deficiency of ^{234}U in the source material, but this is not found in the soil horizon samples (Table 2). The very low activity ratio for the organic matter in stream bedload appears anomalous.

Mangini et al. (1979) and Borole et al. (1982) have shown that river U concentrations vary in proportion to HCO_3^- and total cation concentrations. This is because the major soluble species of U in freshwater of pH >7.5 is $UO_2CO_3^0$ (Langmuir 1978). This transport mechanism is not possible in ELA stream waters of pH 5−6.5 and $M_{co_3} < 10^{-8}$ (Table 7) (see Herczeg and Hesslein 1984). According to Dongarra and Langmuir (1980) the dominant aqueous species of U at pH 4−7.5 will be UO_2OH^+, $UO_2(HPO_4)_2^{2-}$, and UO_2F^+. Li et al. (1980) demonstrated that soluble, low molecular weight humic and fulvic acids strongly bind U at pH 5−7. Uranium may be carried in solution in ELA streams in complexation with PO_4 and dissolved organic carbon, and suspended sediment U may be largely sorbed to chitin and cellulose (Galun et al. 1983). This speculation is confounded by the observation that annual fluxes of TDP, DOC, and PC are larger in the East Basin (Table 14), whereas U fluxes are greater in the NW basin (Table 12). The two-fold difference in U weathering rates in the Northwest and East Basin streams (Table 12) is not related to weathering rates for cations or CO_2 species (Table 14) or discharge (Fig. 2,3).

The Th data present an unsolved puzzle. From the soil and bedload data in Table 2, we should expect ^{230}Th stream transport rates to be equal to or greater than U transport, because

TABLE 14. Gross annual flux of major stable elements from the East and Northwest drainage basins of Lake 239 for 1979 and 1980. For more detailed analysis of major element weathering rates, seasonal annual variation, see Brunskill et al. 1971; Schindler et al. 1976; Schindler et al. 1980. Flux is given in 10^3 mol·km^{-2} drainage area per year. Input via rain and snow is available, but is not included in this analysis.

Drainage basin	Area (km^2)	Water discharge 10^3 m^3·yr^{-1}	Ca	Mg	Na	K	DIC[a]	Cl	SO$_4$	DOC[a]	PC[a]	TDP[a]	PN[a]	TDN[a]	PP[a]
						kmol·km^{-2}·yr^{-1}									
East Basin	1.7028														
1979		248	9.69	5.13	10.3	3.28	26.6	3.44	9.57	163	5.23	5.45	0.50	0.053	0.018
1980		304	20.1	9.81	17.7	5.43	48.6	7.11	21.3	243	6.58	7.52	0.49	0.088	0.037
NW Basin	0.5638														
1979		76.2	5.43	3.65	6.17	0.99	28.4	3.14	8.02	123	3.74	3.99	0.12	0.025	0.015
1980		81.5	9.91	5.91	10.1	2.04	40.3	10.1	15.9	204	5.48	4.49	0.35	0.027	0.010

[a] DIC = dissolved inorganic carbon; PC = particulate carbon; TDN = total dissolved nitrogen; PN = particulate nitrogen; TDP = total dissolved phosphorus; PP = particulate phosphorus.

^{230}Th activities are similar to or less than ^{234}U activities in these source materials. Suspended sediment, however, is depleted in ^{230}Th (relative to ^{234}U, Table 2), and stream water transport of ^{230}Th is an order of magnitude less than for ^{234}U (Table 12). There must be either an unsampled reservoir of ^{230}Th in the drainage basins, or an unmeasured means of ^{230}Th loss from the soils. Some of the missing Th may be in the glacial silt and clay in Lake Agassiz sediments, 160 km to the west. ELA soils are generally built upon sand and gravel deposits, the silt and clay fraction having been sorted and carried away by river and lake erosion in early post-glacial time. Coarse particulate organic matter in bedload is somewhat enriched in Th (Table 2), but we do not have annual flux data for this fraction of bedload. The excess ^{228}Th shown in Table 2 may be derived from the α-recoil ejection and decay of ^{228}Ra and ^{228}Ac from deeper soil groundwater, and subsequent sorption of ^{228}Th to surface soils. Michel (1984) also observed excess ^{228}Th in weathered granites and soils. To improve our estimate of Th transport rates to the lake, we will have to have better measurements of suspended and bedload transport, because much of the Th supply to the lake is in particulate form (Table 10). Thorium transport in the NW Basin (Table 12) is largely in the "dissolved" fraction, however, and Th may be transported largely in complexation with dissolved organic matter, and as $Th(SO_4)_2^0$ and $Th(HPO_4)_3^{2-}$ over the pH range 4–7 (Langmuir and Herman 1980). Wahlgren and Orlandini (1982) also proposed that Th is mobilized by dissolved organic carbon in low pH waters.

The annual flux of "dissolved" ^{226}Ra from the drainage areas of these ELA streams is about an order of magnitude greater than that of its parent ^{230}Th (Table 12), but the source materials (soils, bedload) do not show any depletion of ^{226}Ra (Table 2). From other work on ^{226}Ra at ELA (Emerson and Hesslein 1973, Hesslein and Slavicek 1984), we expect ^{226}Ra to be sorbed to and carried by organic matter, yet the coarse organic bedload material (Table 2) is not enriched in ^{226}Ra. Suspended sediments of stream water are enriched in ^{226}Ra, but this constitutes a minor fraction of the annual flux of Ra to the lake. Hesslein and Slavicek (1984) demonstrated that ^{226}Ra is released from lake sediments under periods of anaerobic conditions. It may be that much of the year's ^{226}Ra flux is derived from poorly oxygenated shallow groundwater flow below the sampled soil horizons. Radium is thought to be carried in fresh waters of pH 4–6 as a $RaSO_4^0$ ion pair and in complexation with humic and fulvic acids (Benes 1982). ELA runoff has very low concentrations of $SO_4^=$ (Tables 7 and 14) but high concentrations of dissolved organic matter. Nathwani and Phillips (1979 a–d) studied Ra leaching and sorption, and found that there were two mechanisms of Ra release from soils, mine tailings and rocks: (1) desorption of Ra from surfaces of particles, a fast reaction and (2) dissolution and diffusion of Ra from host particles and minerals, a slower reaction. These authors proposed that Ra leaching occurs by simple exhange of $2H^+$ for Ra^{+2}, and they demonstrated a minimum leaching rate at pH 7.6, with increasing Ra release rates above and below this pH. Organic matter, FeOOH, and kaolinite are common in ELA soils and sediments, and are the major sorption sites for Ra (Benes 1982; Brunskill et al. 1971). Sorbed Ra is usually more abundant in the fine fraction of soils and sediments (Moffett and Tellier 1977; Megumi 1979; Landa 1984) and often 50–80% of total Ra can be extracted with dilute acid. In the absence of competing cations (such as Ca^{+2}, see Nathwani and Phillips 1979b) and anions for complexation ($SO_4^=$ and $CO_3^=$), and the abundance of organic matter and FeOOH in ELA soils (Table 1), it is perhaps not surprising that the Ra distribution coefficients for water:suspended sediments given in Table 11 are higher than those given by Benes (1982).

TABLE 15. The ratio of Ca to ^{226}Ra in the weathering cycle of Lake 239 bedrock, soils, stream bedload, stream water, lake water, and lake sediments. The ratio was calculated as mol Ca·g^{-1} ÷ Bq ^{226}Ra·g^{-1}.

Sample	Ca: ^{226}Ra (mmol:Bq)	Number of samples
Bedrock	12	6
NW and East Basin soils	13–45	8
Stream bedload	21–32	4
Stream runoff, NW Basin	27–63 ($\bar{X}$ = 48)	17
Stream runoff, E. Basin	74–144 ($\bar{X}$ = 100)	13
Lake 239 surface water	173	10
Lake 239 sediments at 29 m	1–3	3

The group IIa elements Ca and Ra should follow a similar pathway (Justyn and Stanek 1974; Hesslein and Slavicek 1984). In Table 15 we show the relative abundance of Ca and Ra in the weathering cycle at ELA. Ca:Ra ratios (mmol/Bq) are around 12 in bedrock, and increase to over 100 from soils to runoff to lake water. The lake sediments are enriched in Ra, while most of the Ca is lost to outflow. Nathwani and Phillips (1979b) have shown that higher concentrations of Ca will decrease the sorption of Ra onto particulate material. Calcium concentrations and annual fluxes for East Basin runoff are nearly twice those of the NW Basin (Tables 7 and 14), but ^{226}Ra fluxes are not greatly different. However, Ca:Ra ratios within each stream showed little variation: NW Basin 1979, Ca:Ra = 46 ± 12; 1980 = 49 ± 14; East Basin 1980 Ca:Ra = 100 ± 26. Like Descamps et al. (1982), we found that most of the ^{226}Ra was in the solution phase, and that there was little relation of ^{226}Ra activity to water discharge. The annual transport of ^{226}Ra from the Precambrian Shield drainage area of the Serpent River in Ontario (which is the location of several uranium mines and many trailings ponds) is 100–180 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$, three orders of magnitude greater than the values in Table 12 (see Ross and Chatterjee 1976; Ophel et al. 1983).

Many authors have found that ^{210}Pb is firmly fixed to soil and sediment particles, and is not significantly transported by streams (Nozaki et al. 1978; Turekian et al. 1977; Lewis 1976). Our data indicate that of the ≈80 Bq $\mathrm{^{210}Pb \cdot m^{-2} \cdot yr^{-1}}$ supplied by atmospheric fallout to the terrestrial drainage area (Table 4), only 0.7–3 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$ (<3%) was transported by the Northwest and East Basin streams to the lake. Much of this stream transport of ^{210}Pb was in particulate form (Tables 10 and 12) and the annual transport rate was similar to that found by Benninger (1978) for the drainage area of Long Island Sound (≈5 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$). The major source of ^{210}Pb to the lake was from direct atmospheric fallout on the lake surface area. Stream inputs of ^{210}Pb and ^{222}Rn were small (<15% of the total).

Atmospheric ^{210}Pb is apparently supplied to the soil and lake surface as an aerosol according to most studies (Turekian et al. 1977; Peirson et al. 1966; Duce et al. 1976), but Francis et al. (1970) and Ter Haar et al. (1967) found most of their Pb and ^{210}Pb in the "solution" phase. In ELA stream runoff, ^{210}Pb may be carried in solution as $^{210}\mathrm{Pb}^{++}$, $^{210}\mathrm{PbOH}^{+}$, $^{210}\mathrm{Pb(OH)}_2^{\circ}$, *n*-methyl-^{210}Pb and other complexes with dissolved organic matter (Gibson 1961; Harrison and Lazen 1980; Rickard and Nriagu 1978; Chow 1978), and most of the NW Basin ^{210}Pb flux was in this "solution" phase (Table 12).

Lead-210 is greatly enriched in the organic A horizons of the soil profiles and in stream suspended sediments, but shows no enrichment in inorganic materials, such as stream bedload sands (Table 2). Surface soil ^{210}Pb activities are 3–200 times greater than grandparent ^{226}Ra activities, and this excess ^{210}Pb (26–111 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$, Table 4) is likely from the atmospheric decay of ^{222}Rn to ^{210}Pb and subsequent fallout (≈80 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$) to the land and water surface. This atmospheric ^{222}Rn is derived from exhalation of Rn from porous soils; unfissured bedrock and water-covered areas yield little ^{222}Rn to the atmosphere (Turekian et al. 1977). For ELA soils to be both a source and a sink for ^{210}Pb, there should be a deficiency of ^{210}Pb (relative to ^{226}Ra) in the B and C horizons of the soil profiles to match surficial soil excess ^{210}Pb. This deficiency should represent the loss of soil ^{222}Rn to the atmosphere, and it should be equal to the rain plus dry fallout ^{210}Pb flux and/or the surface soil calculated ^{210}Pb fluxes (Table 4). One of our soil profiles shows a deficiency of ^{210}Pb relative to ^{226}Ra in the B and C horizons of NW3 (Table 2), which is equivalent to 38 Bq $\mathrm{^{210}Pb \cdot m^{-2} \cdot yr^{-1}}$. The calculated excess ^{210}Pb flux at the top of this same soil profile is 26 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$, so it appears that approximate equilibrium is maintained at this site. The other soil profiles do not show evidence of ^{222}Rn (or ^{210}Pb) loss, but these East Basin sites (EB1, EB2) have greater than 12 m thicknesses of silt, sand, gravel, and boulders (Beaty 1981, p. 324 and 333). Our soil profiles were only 15–30 cm deep. These thick glacial sediments are likely waterlogged for much of the year, however, and we expect ^{222}Rn diffusion from >1 m soil depth to be negligible. The calculated ^{222}Rn soil flux above (NW3) is not sufficient to maintain the ^{210}Pb atmospheric fluxes shown in Table 4. Much of the surface area of ELA is exposed bedrock, lakes, and water-saturated marshes, swamps, and bogs which will release little ^{222}Rn (Turekian et al. 1977). Perhaps some of the atmospheric ^{222}Rn is derived from drier agricultural soils to the west and south of ELA, where greater soil depth and porosity allow larger ^{222}Rn exhalation. Fleischer (1980b) measured ^{222}Rn fluxes from New Mexico and New York soils, and found the equivalent of a ^{210}Pb deficiency of 250–440 $\mathrm{Bq \cdot m^{-2} \cdot yr^{-1}}$ in the deeper horizons. Nozaki et al. (1978) also found that calculated ^{222}Rn fluxes from soils were much less than the measured excess ^{210}Pb at the soil surface.

The annual flux of ^{210}Po from the stream basins was about a factor of 2–3 greater than its grandparent ^{210}Pb (Table 12). Along with ^{210}Pb and the Th isotopes, ^{210}Po occurred largely in the particulate phase (Table 10), although half of the annual transport rate was in the "dissolved" phase. The environmental chemistry of ^{210}Po is not well known, but Figgins (1961) suggests that the stable state of Po in solution should be tetravalent, but that high concentrations of dissolved organic matter would likely reduce Po (IV) to Po (II), a more soluble and mobile species. The speciation may include $\mathrm{PoO(OH)_2^0}$ and $\mathrm{Na_2PoCl_6^0}$. Polonium forms a colloid in neutral and weakly acidic solutions, and forms very insoluble precipitates with $\mathrm{S^{=}}$, FeOOH, and silicate materials (Figgins 1961). Despite these severe limitations to stream transport, the measured ^{210}Po fluxes are greater than the fluxes of the supposedly more soluble U isotopes (Table 12). This observation implies that ^{210}Po must have some solution transport mechanism that is not available to ^{210}Pb (and the other ^{238}U daughters) and/or that $^{210}\mathrm{Po}:^{210}\mathrm{Pb}$ ratios in precipitation must be greater than 1. Most studies have found $^{210}\mathrm{Po}:^{210}\mathrm{Pb}$ ratios in precipitation and air to be <0.5 (Peirson et al. 1966; Francis et al. 1970; Parfenov 1974; Moore et al. 1976).

If our sample of the granodiorite bedrock at ELA is representative of the parent material of the soils, then there has been considerable leaching of the ^{238}U and ^{232}Th series nuclides from the soil B and C horizons (Table 2). It is also possible that these glacially distributed sands and gravels are derived from Precambrian Shield bedrocks to the north and east of ELA which may have one-half to one-third of the nuclide activity of the Lake 239 bedrock sample. If the latter case is accepted, then there has been considerable sorption and accumulation of U and ^{226}Ra in the organic A horizons of some of the soils and in suspended sediments of the stream. Future studies on the easily leachible fraction of the soil samples might help resolve this question. Michel (1984) studied soil profiles developed upon a parent granitic bedrock, and was forced to propose U and Ra sorption to the soil in order to explain the deficiency of Th in the soil. Based upon Michel's (1984) work, we propose that all U and Ra in excess of the ^{230}Th activity in the soils will be water-born U and Ra that has been sorbed to soil particle surfaces. Rosholt et al. (1966) also found excess U fixation in the organic matter-rich surface horizons of soil.

Conclusions

1. The annual flux of U, Th, Ra from land drainage areas to

streamflow for the Experimental Lakes Area is within the range of measured fluxes for large rivers of the world.

2. The low concentrations of all inorganic ions and suspended sediments, and low pH in these Precambrian Sheild runoff waters preclude many common transport mechanisms for the uranium series nuclides. We speculate that relatively high concentrations of dissolved organic matter in these streams are the dominant means of nuclide transport in the solution phase.
3. For the range of sampled flows in this study, seasonal water flow rates do not appear to control measured activities of U-series nuclides in stream water; therefore, the annual flux of nuclides from these watersheds will be proportional to annual stream discharge.
4. Average solution $^{234}U:^{238}U$ activity ratios for stream runoff were between 0.9 and 1.3, but extreme activity ratios of 0.4 to 2.6 were found in some samples. Differential transport of uranium isotopes is highly variable in these headwater streams, compared to large rivers.
5. Uranium is not likely carried in solution as the carbonate complex in these streams, but may be complexed with dissolved organic matter and PO_4. Uranium was not released from soils in proportion to the weathering of major cations or alkalinity.
6. Low transport rates for Th are probably caused by the small amount of suspended sediments in these streams.
7. Radium is transported by stream flow in porportion to Ca^{+2}, but the ratio Ca : Ra is different for the two streams. Annual ^{226}Ra flux here is three orders of magnitude less than in the region of Elliott Lake uranium mining.
8. The major supply of ^{210}Pb to the lake is from atmospheric fallout in precipitation on the lake surface area. ^{210}Pb supply from stream runoff and ^{222}Rn decay in stream and lake water may account for $<15\%$ of total lake supply.
9. There is an insufficient deficit of $^{222}Rn-^{210}Pb$ in the deeper soil profiles to balance the excess ^{210}Pb in the surface soils and lake sediments. We speculate that atmospheric ^{222}Rn is supplied to this region from the cultivated dry, porous soils of the prairies to the west and south of ELA.
10. Suspended sediment : stream water partition coefficients for U were around 2×10^4, Th ($\approx 4-10$) $\times 10^4$, Ra varied greatly from $(2-20) \times 10^4$, and ^{210}Pb and ^{210}Po had coefficients of $(5-33) \times 10^4$. Stream suspended sediment concentrations were low ($<1-10$ $g \cdot m^{-3}$).

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Determining Sediment Accumulation and Mixing Rates Using ^{210}Pb, ^{137}Cs, and Other Tracers: Problems Due to Postdepositional Mobility or Coring Artifacts

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Anderson, R. F., S. L. Schiff, and R. H. Hesslein. 1987. Determining sediment accumulation and mixing rates using ^{210}Pb, ^{137}Cs, and other tracers: problems due to postdepositional mobility or coring artifacts. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 231–250.

Sediment profiles of ^{137}Cs in 12 lakes and of radionuclides (^{60}Co, ^{134}Cs, ^{226}Ra) added experimentally to four lakes at the Experimental Lakes Area, northwestern Ontario, were inconsistent with sediment chronologies derived from ^{210}Pb distributions. Peaks in the ^{137}Cs profiles were sometimes shallower than the depth corresponding to the 1963 fallout maximum (based on ^{210}Pb chronology) and sometimes ^{137}Cs peaks were not observed at all. Expected peaks in the profiles of experimentally added nuclides were similarly absent in three of four lakes. The absence of these peaks at depths corresponding to their time of maximum input to the lakes probably resulted from the initial uptake of the nuclides by littoral sediments followed by the winnowing over periods of several years of nuclide-tagged fine particles to the deeper parts of the basins where the cores were collected. Sediment focussing is manifested by ^{210}Pb sediment inventories at the coring sites greatly exceeding inventories that can be supported by atmospheric deposition of ^{210}Pb in this area.

^{137}Cs and the experimentally added nuclides were found much deeper in the cores than the depths corresponding to the time of their initial inputs to the lakes based on ^{210}Pb chronologies. Diffusion of the nuclides in the pore waters could explain some of their downward penetration. However, in some cases, the profiles could only be fit if the effective diffusion coefficient increased with depth. This situation might reflect mixing of these high porosity sediments during core collection or extrusion.

Distributions of ^{137}Cs and of the experimentally added nuclides do not provide valid chronologies which can be used to date other features recorded in the sediments of these lakes. Although unsupported ^{210}Pb profiles in these lakes provided excellent fits to classical steady-state sediment accumulation models, ^{210}Pb profiles too may have been affected by sediment mixing or core disturbance. Further studies are necessary to test the reliability of ^{210}Pb-based sediment chronologies in high porosity sediments such as those studied at ELA.

Les profils sédimentaires de ^{137}Cs dans 12 lacs et de radionucléides (^{60}Co, ^{134}Cs, ^{226}Ra) ajoutés expérimentalement à quatre lacs dans la Région des lacs expérimentaux, au nord-ouest de l'Ontario, ne concordaient pas avec les chronologies sédimentaires tirées des distributions de ^{210}Pb. Les pics dans les profils de ^{137}Cs ont été parfois moins profonds que la profondeur correspondant au maximum de retombées (basées sur la chronologie du ^{210}Pb) et parfois les pics de ^{137}Cs n'ont pas été observés du tout. Les pics prévus dans les profils de nucléides ajoutés expérimentalement étaient de même absents dans trois lacs sur quatre. L'absence de ces pics à des profondeurs correspondant au moment où leur apport aux lacs a été maximal résultait probablement de l'adsorption initiale des nucléides par les sédiments du littoral suivie sur des périodes de plusieurs années par le triage de particules fines marquées de nucléides aux parties plus profondes des bassins où on recueille les carottes. La concentration des sédiments se manifeste par des inventaires de ^{210}Pb dans les sédiments aux endroits où s'effectue le carottage qui dépassent de beaucoup les inventaires qui peuvent être justifiés par le dépôt atmosphérique de ^{210}Pb dans ce secteur.

Le ^{137}Cs et les nucléides ajoutés expérimentalement ont été découverts à une profondeur beaucoup plus grande dans les carottes que les profondeurs correspondant au moment de leurs apports initiaux aux lacs à partir des chronologies du ^{210}Pb. La diffusion des nucléides dans les eaux des pores pourrait expliquer une certaine partie de leur pénétration vers le bas. Cependant, dans certains cas, les profils ne pouvaient être ajustés que si le coefficient de diffusion réel augmentait avec la profondeur. Cette situation pourrait refléter le mélange de ces sédiments à porosité élevée lors de la collecte ou du prélèvement des carottes.

Les distributions de ^{137}Cs et des nucléides ajoutés expérimentalement ne fournissent pas des chronologies valables qui peuvent être utilisées pour dater d'autres entités enregistrées dans les sédiments de ces lacs. Bien que les profils de ^{210}Pb non étayés dans ces lacs aient fourni d'excellents ajustements aux modèles classiques des

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sédiments à l'état stable, les profils de ^{210}Pb auraient pu être affectés également par le mélange des sédiments ou la perturbation des carottes. D'autres études sont nécessaires pour vérifier la fiabilité des chronologies à base de ^{210}Pb dans des sédiments à forte porosité tels que ceux étudiés dans la RLE.

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The use of the naturally occurring radionuclide ^{210}Pb to determine sediment accumulation rates in marine and freshwater sediments is now a well-established technique. The principles, assumptions, and limitations of the method are described in early papers introducing the procedure (Krishnaswami et al. 1971; Koide et al. 1972; Robbins and Edgington 1975) and in a review by Robbins (1978). After production in the atmosphere by the decay of gaseous ^{222}Rn, ^{210}Pb is deposited on the earth's surface with aerosol particles (Turekian et al. 1977). ^{210}Pb introduced into aquatic systems is rapidly incorporated into settling particles and buried in the sediments. It is the decay of this ^{210}Pb which is unsupported by its parents in the ^{238}U decay series that provides a measure of time in the sedimentary record (see above references).

Radionuclides originating as fallout from nuclear weapons testing have also been used to obtain sediment chronologies. The peak fallout activity of ^{137}Cs and $^{239+240}$Pu in 1963 has become an often-used stratigraphic marker (e.g. Robbins and Edgington 1975; Pennington et al. 1976; Durham and Joshi 1980; Olsen et al. 1981; Jaakola et al. 1983). To verify the sediment chronologies determined from ^{210}Pb, several investigators have measured the depth distributions of multiple tracers. Robbins and coworkers (Robbins and Edgington 1975; Robbins et al. 1977) pointed out that ^{137}Cs occurred too deep in Great Lakes sediments to be compatible with ^{210}Pb chronologies unless the mixing of surface sediments by benthic organisms was taken into account.

The distribution of a tracer within a sediment column is determined by its history of input to the lake, by its rate of removal to the sediments, by redistribution of the sediments within the lake, by the rates of sediment accumulation and of sediment mixing (e.g. bioturbation) and by diffusion in pore waters. Tracer distributions would also be affected, of course, if there were mixing or smearing of the sediments during core collection. The relative effect of these processes on the profile of a tracer cannot be resolved from the distribution of a single radionuclide. Sediment distributions of two or more radionuclides with different half lives, or with different input histories, must be used to overcome this difficulty (e.g. Robbins and Edgington 1975). In most past studies only two radionuclides, or radionuclides covering only two time scales, have been considered. A unique opportunity to use additional discrete stratigraphic markers is provided at the Experimental Lakes Area where three whole lakes have been spiked with gamma-emitting radiotracers. Two of these three lakes, and a fourth lake, were also spiked with ^{226}Ra. The depth distributions of the nuclides ^{134}Cs, ^{60}Co, and ^{226}Ra in the spiked lakes as well as the distributions of ^{210}Pb and ^{137}Cs in these and several other lakes were measured in an attempt to obtain accurately constrained rates of sediment accumulation and mixing in these typical Canadian Shield lakes.

Sampling Locations and Methods

The Experimental Lakes Area is located on the Canadian Shield in northwestern Ontario. The reader is referred to a number of earlier publications for descriptions of the biology, chemistry, and geology of the lakes (Brunskill and Schindler 1971; Brunskill et al. 1971; Schindler 1971; Armstrong and Schindler 1971). Lake 224 was spiked with a suite of gamma-emitting radiotracers in 1976 (Hesslein et al. 1980). The North and South subbasins of Lake 226, which were separated by a plastic curtain, were spiked in 1977 (Hesslein 1987). ^{134}Cs and ^{60}Co were included in both of these experiments. In addition, ^{226}Ra was added to L224 in 1976, to the south basin of L226 in 1978 (Hesslein, unpublished data) and to L227 in 1970 (Emerson and Hesslein 1973). Each of these nuclides was removed from the water column to the sediments of these lakes within a few weeks after spiking (Emerson and Hesslein 1973; Hesslein et al. 1980; Hesslein 1987).

These four lake basins and Lakes 114, 222, 223, 239, 240, 302N, and 302S were sampled near their deepest point (except L240, which was sampled from a moored raft near the north end of the lake at a water depth of about 9 m) in 1980. An additional core was taken from the deepest part of the south subbasin of L259 in 1982. Cores were collected with a large diameter ($\simeq$15 cm) modified piston corer. The corer was lowered slowly to the sediment–water interface where contact with the sediments interrupted a beam of light directed at a photocell. This released a weighted plexiglass core barrel allowing it to slide down around the piston which remained stationary $\simeq$25 cm above the sediment–water interface. The corer was then raised slowly to the surface where a plug with a double O-ring seal and a plastic cap were placed over the bottom of the core barrel before the core was completely removed from the water. The core barrel was detached from the rest of the apparatus, visually inspected for disturbance, sealed, and returned to the field camp while always maintained in a vertical position. There, the core barrel was clamped to a vertical holder and sediments were extruded upward with the plug on a calibrated screw-driven piston. This corer and handling procedure were specifically designed to collect a well preserved sediment–water interface in the loose, flocculent, organic-rich sediments that accumulate in the deepest parts of ELA lakes.

Wet sediments were placed in plastic bags and shipped to Lamont–Doherty Geological Observatory where they were freeze-dried and sealed in aluminum cans for gamma counting on a Ge(Li) detector coupled to a 4096-channel pulse height analyzer. The detector efficiency was determined by counting standards in the same geometry configuration as the samples. Activities of ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra (through its decay product ^{214}Bi) were determined by gamma counting. Further details of these procedures can be found in Olsen et al. (1981).

Subsequent to gamma counting, aliquots of each sediment sample were analyzed for ^{210}Pb as follows: 0.25–2.0 g of dried sediment (depending on depth in the core) were weighed in a teflon beaker, spiked with a known amount of ^{208}Po, and dissolved using HCl, HNO_3, HF, and $HClO_4$. Samples were diluted to about 50 mL with 0.5N HCl and Fe(III) was reduced to Fe(II) with ascorbic acid. Po isotopes were spontaneously plated on 2 cm diameter silver discs at a temperature between

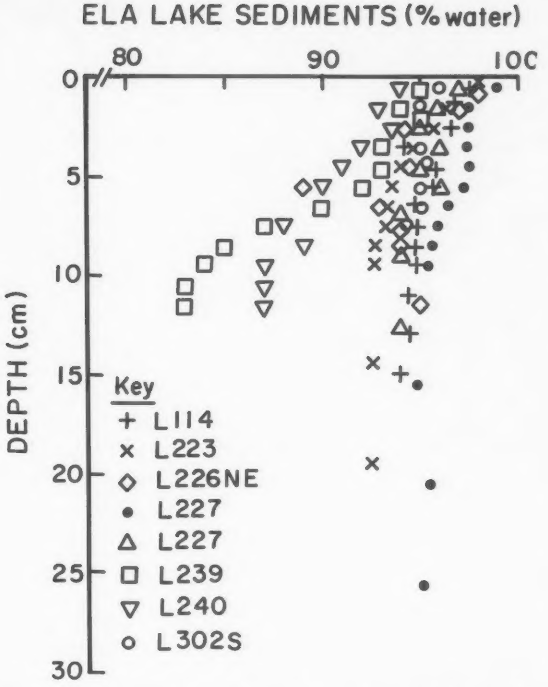


FIG. 1. Water content (percent weight loss on drying) of ELA lake sediments. Data are from: L114 (Anderson, unpublished data); L223 and L227 (solid circle) (Cook 1981); all others (Kipphut 1978).

90 and 95°C. The discs were counted within a few days of plating on silicon surface barrier detectors coupled to a multiplexed pulse height analyzer with 512 channels of memory per detector. The activity of ^{210}Po, the granddaughter of ^{210}Pb, was calculated from the ^{210}Po/^{208}Po activity ratio and the known amount of ^{208}Po added. Samples were processed for ^{210}Pb (^{210}Po) more than 18 mo after collection, so equilibrium between ^{210}Po and ^{210}Pb can be reasonably assumed.

Activities of ^{210}Pb unsupported by its parent ^{226}Ra (xs^{210}Pb) were calculated assuming that the ^{226}Ra-supported ^{210}Pb activity was uniform in each core, and that the constant ^{210}Pb activity at the bottom of each core represented ^{210}Pb in equilibrium with ^{226}Ra. For these deep samples the ^{226}Ra measured by gamma counting agreed with ^{210}Pb values within the uncertainty of the counting statistics. However, for the shallower depths in these cores, sample sizes were too small (often <2 g) to obtain good precision for ^{226}Ra by gamma counting. Radium concentrations could not have been much higher in surface sediments than at depth or the radium would have been detected. The estimated uncertainty in the ^{226}Ra-supported ^{210}Pb activity, derived either from the sample to sample variability in the deep samples of a given core, or, from the lake to lake variability for cores which had only one sample deep enough that we are confident that it contained no xs^{210}Pb, has been incorporated into the propagation of error calculations for xs^{210}Pb values reported in this paper.

Excess ^{226}Ra activities for the sediments of L226S and L227 were similarly calculated by assuming that the constant ^{226}Ra activity deep in each core represented the natural concentration which could be extrapolated to the core top.

Because of concern for the possible importance of diffusive transport of ^{137}Cs and other nuclides in pore waters, a large volume grab sample of surface sediments was collected from near the deepest point of L226N. About 20 cm of surface sediments and 2–3 cm of overlying bottom water were recovered in an Eckman grab sampler. The sample was quickly transferred into six 250 mL plastic centrifuge bottles filled to the top and capped tightly to exclude air as much as possible. After centrifuging, one bottle was opened at a time and the supernate was filtered as quickly as possible, first through a glass fiber filter and then through a Millipore 0.45 μm pore size filter. Colloidal radionuclides may therefore be included in the pore water sample. One liter of pore water was obtained in this manner and acidified with HCl. Total processing time (after sample collection) was about 6 h. Centrifuged sediments were dried at 60°C and saved for analysis. The acidified pore water was spiked with a ^{208}Po tracer and a stable Pb carrier and evaporated in a teflon beaker with addition of HNO_3 to digest dissolved organic matter to a final volume of ≃ 5 mL. It is assumed that recovery of the gamma-emitting isotopes was quantitative. The solution was gamma counted for ^{134}Cs, ^{137}Cs, ^{60}Co, and ^{226}Ra by the same procedure that was used for sediment samples, after which the sample was set aside for several months before processing for ^{210}Pb(^{210}Po) by the same procedure that was used for sediment samples.

Sediment porosities of the cores used for this study could not be measured accurately because a small, but unknown, amount of water leaked from the plastic bags in transit to Lamont. Porosity measurements have been made on other cores from these lakes which show that typically the water content by weight is very high, ranging between 95 and 98% at the surface (Fig. 1). Except for Lake 114, all of the cores for which water contents are shown in Fig. 1 were collected within about 20 m of where the cores were collected for this study. The data shown in Fig. 1 were therefore used to calculate porosities (Φ) for these lakes (223, 226NE, 227, 239, 240, 302S) according to

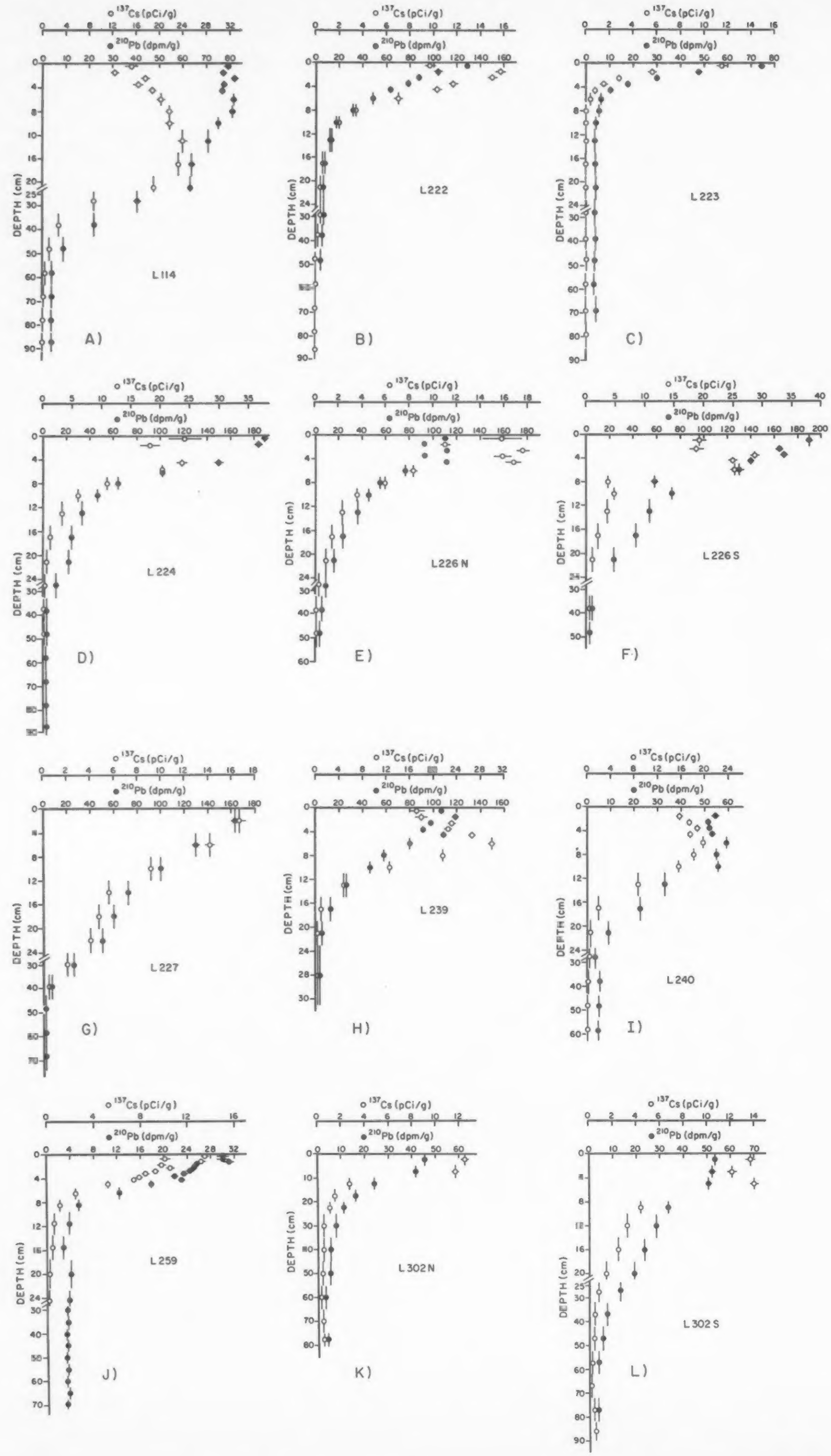
$$(1) \quad \Phi = \frac{\%W}{\%W + (1 - \%W)/\rho_s}$$

where $\%W$ is the measured percent water by weight and ρ_s is the dry density of the sediments which was assumed to be $2.5\ \mathrm{g \cdot cm^{-3}}$. For the other lakes, the following assumptions were made: L114 — the porosity of a core collected at 1.2 m water depth with similar sediment consistency was used; Lakes 222 and 224 were assumed to be similar to L223; L226SW was assumed to be similar to L226NE; L259 was assumed similar to L240; and L302N was assumed similar to L302S.

Dating Methods

Application of the ^{210}Pb dating technique requires the following assumptions: (1) the deposition of ^{210}Pb is at steady state, and (2) there is no postdepositional mobility of ^{210}Pb except for physical or biological mixing of the sediments which is restricted to a narrow zone near the sediment–water interface. If these assumptions hold, then the distribution of xs^{210}Pb in the sediments can be represented as

$$(2) \quad \frac{\partial \rho A}{\partial t} = \frac{\partial}{\partial z} \cdot \frac{D_B \partial \rho A}{\partial z} - \frac{S \partial \rho A}{\partial z} - \lambda \rho A$$


○ ^{137}Cs (pCi/g)
● ^{210}Pb (dpm/g)
DEPTH (cm)
L114
A)
L222
B)
L223
C)
L224
D)
L226 N
E)
L226 S
F)
L227
G)
L239
H)
L240
I)
L259
J)
L302 N
K)
L302 S
L)

where:

A = the activity of xs^{210}Pb (dpm·g^{-1})
t = time
z = depth
ρ = in situ sediment density (g[dry]·cm^{-3}[wet]) = $(1-\Phi)\rho_s$
D_B = sediment mixing rate (cm^2·s^{-1})
S = sedimentation rate (cm·s^{-1})
λ = radioactive decay constant for ^{210}Pb

This equation is usually simplified by assuming that D_B is constant over the depth of the mixed layer (L), and that the in situ density (or porosity) is uniform with depth. Neither of these assumptions is strictly valid. However, for the purposes of this discussion, these are second order effects because the greatest porosity gradients occurred within the upper 5 cm which included the apparent mixed layer for most of these cores (see below). Sedimentation rates were calculated from ^{210}Pb distributions in sediments below the zone of sharp porosity gradient and below any visible break in the slope of the xs^{210}Pb distributions which are normally interpreted to indicate the base of the mixed layer ($z = L$). If mixing is insignificant below this depth, then for constant porosity and for $z > L$, Equation (2) reduces to

$$(3)\qquad \partial A/\partial t = -S\partial A/\partial z - \lambda A = 0$$

which has the solution

$$(4)\qquad A(z) = A_o \exp[-(\lambda/S)z]$$

where $A(z)$ is the activity at depth (z) and A_o is the activity at the base of the mixed layer, or at the sediment–water interface when no mixed layer exists. Apparent sedimentation rates were calculated for each core by applying Equation (4) to the log-linear portion of the xs^{210}Pb distributions. These rates are upper limits as any mixing below $z = L$ will increase the apparent sedimentation rate above the true value.

For situations where sediment accumulation is negligible and radionuclide profiles are determined entirely by vertical mixing and decay, for constant Φ and D_B Equation (2) reduces to

$$(5)\qquad \partial A/\partial t = D_B \partial^2 A/\partial z^2 - \lambda A$$

which has the solution

$$(6)\qquad A(z) = A_o \exp[-(\lambda/D_B)^{1/2} z]$$

Atmospheric deposition is the dominant source of ^{137}Cs to these lakes. Stream inflow introduced negligible ^{137}Cs to these lakes compared to atmospheric fallout even in recent times (1981–83; S. Schiff, unpublished results) when atmospheric deposition of ^{137}Cs has been very low. Therefore, it is unlikely that stream inflow was ever a significant source of ^{137}Cs to these lakes during earlier years when the atmospheric flux was higher and the inventory of ^{137}Cs in the watershed was less. Significant amounts of ^{137}Cs deposition began to occur about 1953 and maximum fallout levels occurred in 1963 (Hardy 1977; EML 1982). The half time for removal of Cs to the sediments of these lakes by chemical scavenging processes is only 2–4 wk (Hesslein et al. 1980; Hesslein 1987). Therefore, the sedimentary ^{137}Cs profiles should closely match the fallout record if there has been no postdepositional mobility of ^{137}Cs in these lakes, and the first appearance of ^{137}Cs in a core, and the depth of its maximum activity, provide dates of ≃1953 and 1963, respectively. For these cores collected in 1980, significant amounts of ^{137}Cs should not occur at depths much greater than the interval corresponding to one ^{210}Pb half life (1980 − 22 = 1958) unless ^{137}Cs is transported downward by diffusion or sediment mixing.

Experimentally added radionuclides provided an additional constraint on the sediment chronologies. If there was no postdepositional migration of these tracers, then their depths of first appearance could be assigned to the time (within a few weeks) that the tracers were added to the lakes. Penetration of these tracers to greater depths than expected from the ^{210}Pb sedimentation rates and their known input histories provided an extremely sensitive indicator of downward transport by sediment mixing or by diffusion in pore waters.

Results

^{210}Pb Distributions

Concentrations of ^{210}Pb in these cores decreased with depth (Fig. 2) as expected for conditions of continuous ^{210}Pb deposition and sediment accumulation. ^{210}Pb activities eventually levelled off at constant values deeper in the cores, indicative of the ^{226}Ra content of the sediments. Three types of xs^{210}Pb distributions were observed:

1) Most of the cores (Lakes 114, 224, 226NE, 239, 240, 259, and 302S) had uniform ^{210}Pb activities in the upper few centimeters, below which the xs^{210}Pb activity decreased exponentially with depth (Fig. 3). This type of distribution is often observed in sediments and is interpreted to indicate rapid mixing of surface sediments either by biological or physical processes. The presence of a mixed layer in the sediments of L302N, where there is some evidence of a break in the xs^{210}Pb gradient (Fig. 3K), and in L227 (Fig. 3G) may have been obscured by the thicker sampling intervals collected for these cores compared to the other lakes.

2) The xs^{210}Pb distributions in Lakes 114, 226S, and 259 (Fig. 3A, F, and J) suggested a three-layer system which included: (a) a surface layer of rapidly mixing sediments with a uniform xs^{210}Pb content (<1 cm or non existant in L226S), (b) a second layer in which slower mixing was evident in that the xs^{210}Pb content decreased with depth, but less rapidly than deeper in the core, and (c) the deepest layer in which the xs^{210}Pb activity decreased exponentially, and more rapidly, with depth. Similar systems with two mixed layers having different

FIG. 2. ^{210}Pb and ^{137}Cs distributions in sediment cores collected from 12 lake basins at the Experimental Lakes Area. All of the cores were collected at the maximum depth of each lake except for L259, where the core was taken from the deepest point of the south basin, and for L240 from which the core was collected from a moored raft at about 9 m water depth near the north end of the lake. All of the cores were collected in 1980, except for L259 which was collected in 1982. A = L114, B = L222, C = L223, D = L224, E = L226 North Basin, F = L226 South Basin, G = L227, H = L239, I = L240, J = L259, K = L302 N, L = L302 S. Note scale break in the depth axes.

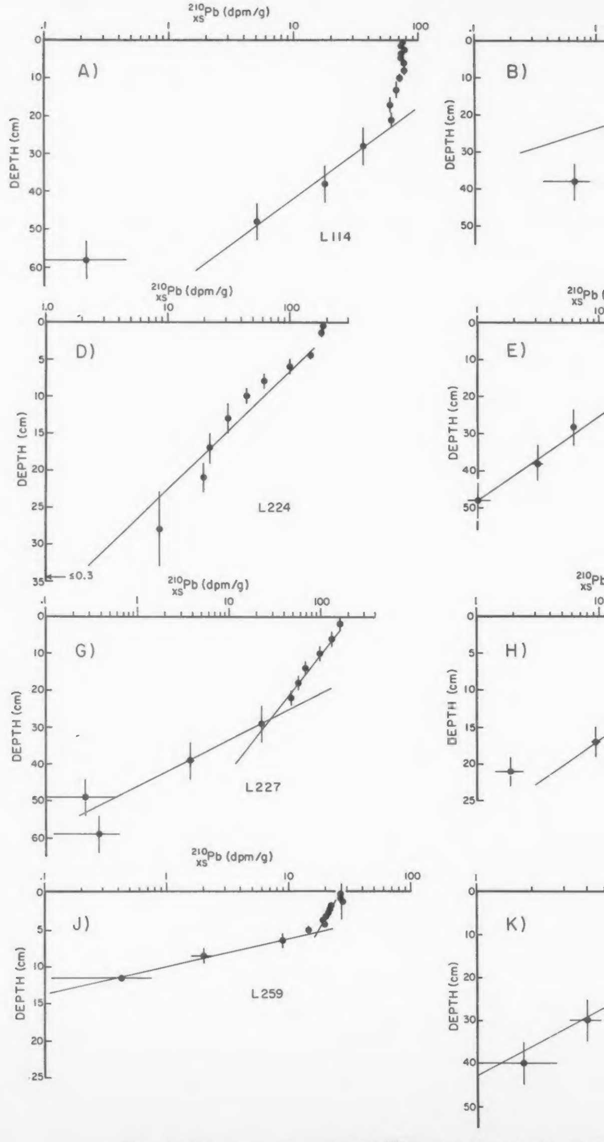
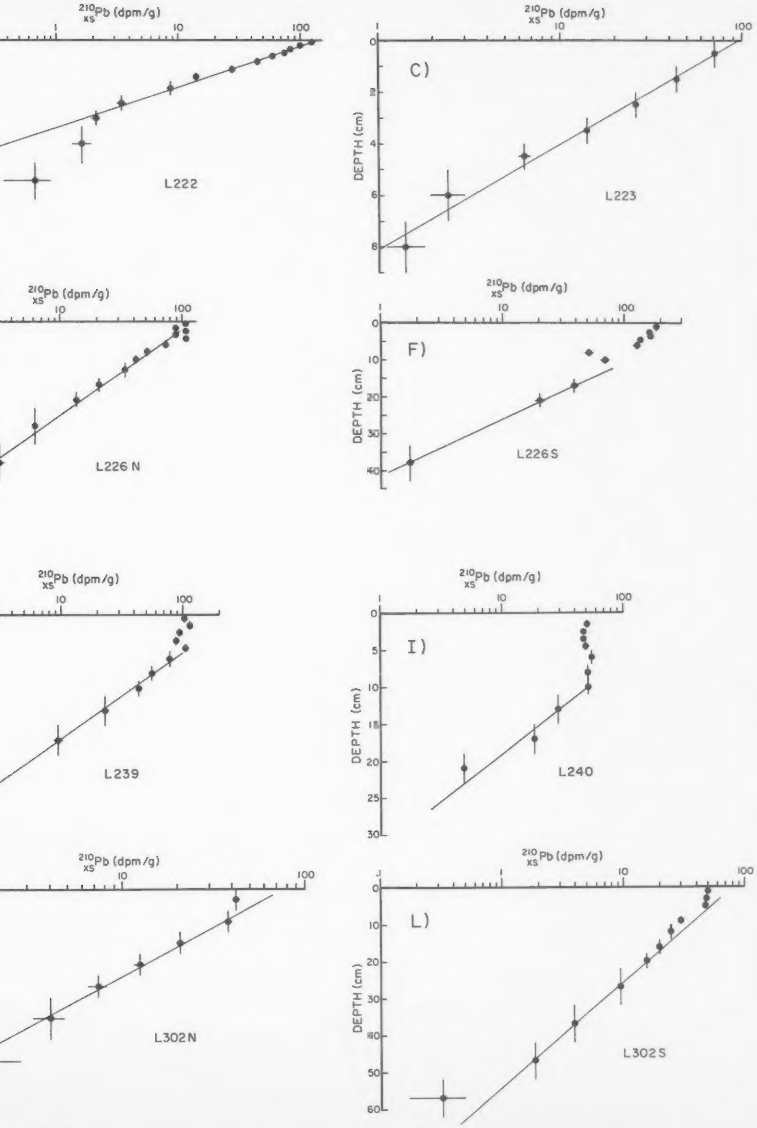


FIG. 3. Downcore unsupported ^{210}Pb activities in the 12 E



the 12 ELA cores. Figure designations A through L are the same as in Fig. 2.

TABLE 1. Summary of the main features of the ^{210}Pb and ^{137}Cs distributions in 12 ELA lakes and other information relevant to interpreting the radionuclide profiles

Lake	Seasonal anoxia at coring site[a]	^{210}Pb mixed layer		^{137}Cs Peak		^{137}Cs	Sediment inventories[b]	
		Present	depth (cm)	Present	depth (cm)	Penetration depth (cm)	xs^{210}Pb (dpm·cm^{-2})	^{137}Cs (mCi·km^{-2})
114	Yes	Yes	0–9 (0–24)?	Yes	11–15	>53	129	359
222	Yes	No	—	Yes	1–2	>33	37	57
223	Yes	No	—	No	—	5–7	6.0	7.2
224	No	Yes	0–5	?	<4	>33	99[c]	121[c]
226NE	Yes	Yes	0–5	Uniform	0–5	>23	75	78
226SW	Yes	Yes[d]	0–7	Yes	3–4	>23	109	120
227	Yes	No	<4	No	<4	>34	103 (87)[e]	98
239	No	Yes	0–5	Yes	5–7	>23	107	287
240	No	Yes	0–11	Yes	5–7	>33	88	274
259	?	Yes[d]	0–5	?	<3	>18	11	46
302N	Yes	Maybe	<5	?	<5	>55	40	97
302S	Yes	Yes	0–6	Uniform	0–6	>42	54	91

[a] Unpublished data reports, D. W. Schindler, Freshwater Institute, Winnipeg, Man.

[b] Sediment porosities could not be determined for these cores. Porosities used to calculate radionuclide inventories were obtained as follows: L114 — assumed similar to other L114 cores for which porosities have been measured by the senior author; L222 — assumed similar to L223; L223 and L227 — porosities were taken from Cook (1981) who reported values for cores collected within a few metres of ours; L224 — assumed similar to L223; L226NE, L239, L240, L302S — porosities were taken from Kipphut (1978) who reported values for cores collected within a few metres of ours; L226SW — assumed similar to L226NE; L259 — assumed similar to L240; L302N — assumed similar to L302S.

[c] Inventories were calculated by interpolating activities for the 2–4 cm depth interval for which the samples were lost.

[d] Mixed layers were evident as a decrease in the slope of the unsupported ^{210}Pb activity with depth, although mixing was not sufficiently rapid to make a uniform ^{210}Pb activity over the depth interval.

[e] Unsupported ^{210}Pb inventory corrected for ingrowth from ^{226}Ra in the sediments that was added to the lake in 1970. An ingrowth period of 8 yr was assumed for all depths. Excess ^{226}Ra (added as a spike in 1978) in the sediments of L226SW was present at less than 10% of the activity of xs^{226}Ra in L227, and consequently ^{210}Pb ingrowth during the 2 yr between spiking and coring was negligible.

mixing rates have been observed by other investigators (e.g. Santschi et al. 1980; Carpenter et al. 1982).

3) Not all cores exhibited a surface mixed layer. The xs^{210}Pb activity decreased exponentially downward from the sediment–water interface with a constant gradient in Lakes 222 and 223 (Fig. 3B and C). This may have resulted from anoxic conditions, which occur in the bottom waters of these lakes during stratification (Schindler et al. 1980; Kelly et al. 1982; Schindler, unpublished data), thereby preventing the establishment of benthic fauna communities which would mix the sediments. However, xs^{210}Pb distributions in other lakes (L114, L226N, L226S, L302N, L302S) which also have seasonally anoxic bottom waters (Table 1) show well mixed xs^{210}Pb distributions in their surface sediments. Therefore, the absence of mixed layers is not simply correlated with seasonal anoxia in bottom waters.

^{137}Cs Distributions

A tracer, such as ^{137}Cs, with a shorter input history than ^{210}Pb, would be a more sensitive indicator of mixing below the zone of uniform xs^{210}Pb content. In no case did we observe a completely uniform ^{137}Cs distribution throughout a ^{210}Pb mixed layer. In Lakes 226N, 259, and 302S the ^{137}Cs activities within the ^{210}Pb mixed layers were variable, but no discrete maxima were evident (Fig. 2E, J, and L). In Lakes 114, 226S, 239, and 240 (Fig. 2A, F, H, and I), ^{137}Cs maxima were clearly evident within, or below, a mixed layer of uniform ^{210}Pb activity.

The most striking feature of the ^{137}Cs distributions was that in every core ^{137}Cs penetrated to the same depth (within analytical uncertainty) as unsupported ^{210}Pb (compare Fig. 2 and 3) over the entire range of penetration depths from <10 cm in Lake 223 to >50 cm in Lake 114. Significant amounts (>3σ above detection limits) of ^{137}Cs were found more than four ^{210}Pb half-lives below the depth of the ^{210}Pb mixed layer, or below the sediment–water interface where no mixed layer was evident, in every core. If no redistribution of ^{210}Pb occurred below the depths of the mixed layers, then sediments at these depths (>4 half-lives of ^{210}Pb) would have been deposited before the turn of the century, more than 50 yr before the introduction of ^{137}Cs into the environment.

The shapes of the ^{137}Cs profiles in the deeper parts of many cores are remarkably similar to the shapes of the corresponding ^{210}Pb profiles (Fig. 2B, C, D, E, G, H, K, and L). When xs^{210}Pb activities are plotted against ^{137}Cs activities (Fig. 4) an unexpected pattern appears repeatedly. Below the bottom of the ^{210}Pb mixed layer or the ^{137}Cs maximum, where either one exists, to the deepest sediments containing xs^{210}Pb, the data points follow one or two distinct trends. The upper line, or tangent to the curve where the points are nonlinear, in each case has a positive xs^{210}Pb intercept whereas the line through the deeper samples intersects close to the origin. A positive xs^{210}Pb intercept is expected in the absence of nuclide redistribution because sediments deposited for several decades prior

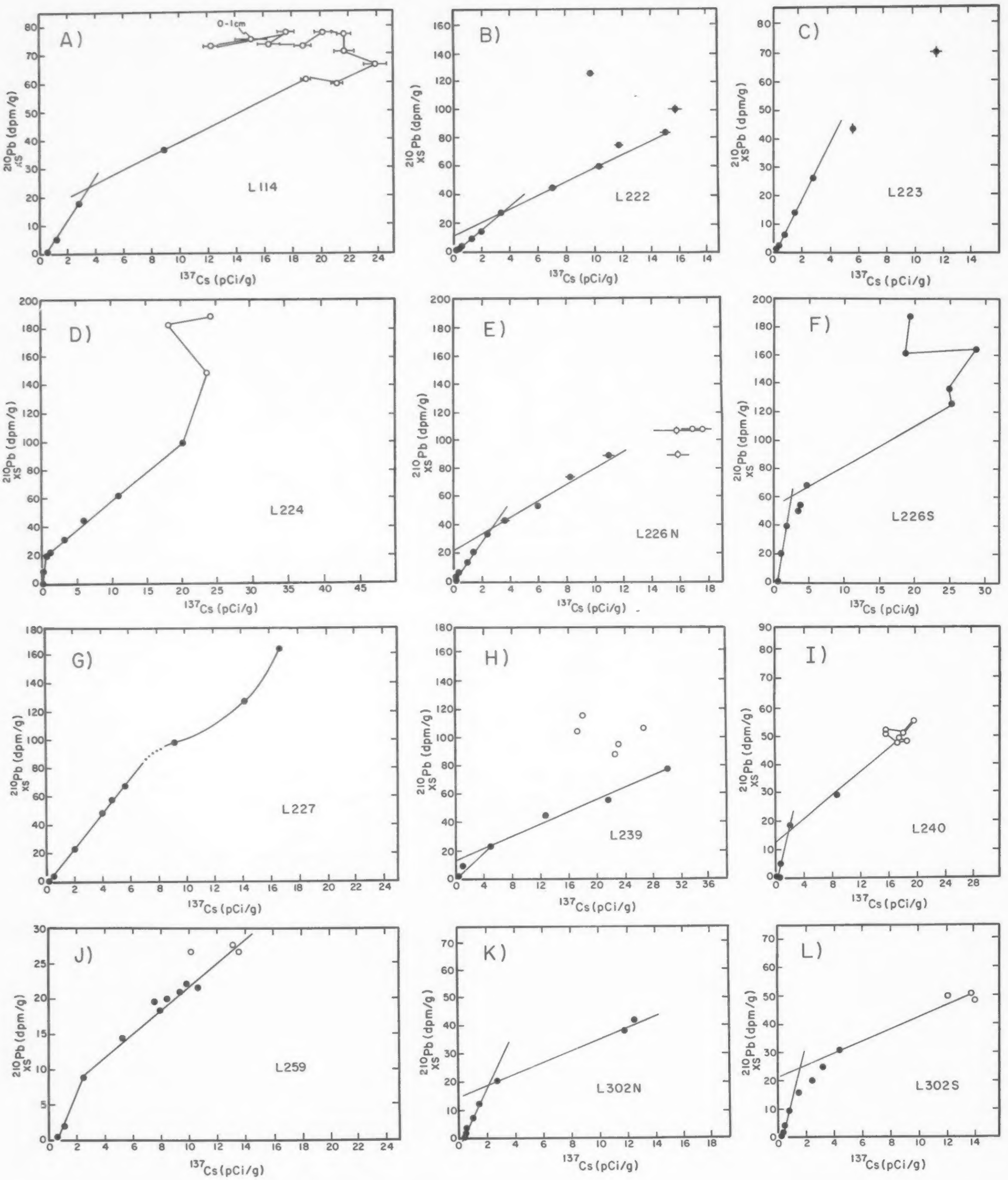


FIG. 4. Unsupported ^{210}Pb activities are plotted against ^{137}Cs activities to illustrate the linear relationship between the distributions of these two radionuclides in the deeper portions of most of the cores. Figure designations A through L are the same as in Fig. 2. Open circles represent points within the mixed layer.

TABLE 2. Benthos in ELA lakes.[a,e,g]

Lake	Chiromonidae[b]	Chaborinae	Amphipoda[c]	Ephemeroptera	Oligochaeta[d]	Nemetoda[d]	Mycidacae
114	Y	N	Y	N	Y	Y	N
222	Y	N	Y	N	Y	Y	N
223	R	N	N	N	N	R	N
224	R	N	N	N	Y	Y	N
226NE	R	Y	N	N	R	R	N
226SW	R	Y	N	N	R	R	N
227	R	Y	N	N	R	R	N
239	Y	N	Y	Y	Y	Y	Y
240	Y	N	Y	Y	Y	Y	Y
259	?	?	?	?	?	?	N
302N	R	N	N	N	R	R	N
302S	R	N	N	N	R	R	N
Mixing depth (cm)[f]	0–2	0–1	Surficial	0–2	0–2	0–2	Surficial

[a] Y = observed, N = not observed in surveys and presumed to be absent, R = rare, ? = unknown.

[b] Chiromonidae densities decrease exponentially with depth. Consequently, densities at maximum lake depth are very low ($\simeq$50–100 emergence of individuals$\cdot$m$^{-2}\cdot$yr^{-1}).

[c] Including pontoporeia, hyalella/gamaras, crangonyx.

[d] Oligochaeta and nemetoda occur only at very low densities in ELA lakes.

[e] Crayfish are restricted to more solid substrates. No live spherid clams have been recovered in ELA lake sediments. Whitefish, suckers and lake trout may also disturb surficial sediments.

[f] Depth interval over which indicated organisms are likely to mix sediments.

[g] Information supplied by Ian Davies, Freshwater Institute, Winnipeg, Manitoba.

TABLE 3. Summary of estimates of sediment accumulation rates for 12 ELA lakes.

Lake	^{210}Pb log-linear[a] $(\text{cm}\cdot\text{yr}^{-1})$	^{210}Pb log-linear[a] $(\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1})$	^{210}Pb As[c] $(\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1})$	^{137}Cs peak depth $(\Delta\tau = 17$ yr) $(\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1})$	^{137}Cs penetration depth $(\Delta t = 27$ yr) $(\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1})$
114	0.30	0.023	0.042	0.025	>0.14
222	0.15	0.0092	NA	0.0029	>0.10
223	0.055	0.0034	NA	NA	~0.011
224	0.21	0.017	0.015	<0.0094	>0.10
226NE	0.27	0.022	0.015	<0.014	>0.056
226SW	0.21	0.016	NA	0.0085	>0.056
227	0.43	0.021	NA	<0.006	>0.081
239	0.18	0.028	0.023	0.024	>0.13
240	0.17	0.026	0.020	0.028	>0.17
259	0.05[b]	0.0056[b]	0.010[b]	<0.009	>0.069
302N	0.31	0.023	0.022	<0.015	>0.16
302S	0.38	0.028	0.024	<0.018	>0.12

[a] Accumulation rates in $(\text{cm}\cdot\text{yr}^{-1})$ are taken from the log-linear portions of the unsupported ^{210}Pb profiles, and correspond to the lines drawn in Fig. 3. Accumulation rates in $(\text{g}\cdot\text{cm}^{-2}\cdot\text{yr}^{-1})$ were calculated using porosity values as described in the footnote of Table 1.

[b] The ^{210}Pb distribution in this core showed 2 zones with different apparent mixing rates located above the interval interpreted to give the ^{210}Pb sedimentation rate (5–10 cm). Sedimentation rates based on the log-linear ^{210}Pb distribution are poorly constrained in this core.

[c] Sediment accumulation rate calculated from the mixed layer ^{210}Pb activity and the inventory of ^{210}Pb in the core as described in the text (Eqn. 15).

to the onset of nuclear weapons testing should contain no ^{137}Cs and easily measurable levels of xs^{210}Pb. In no case was a positive xs^{210}Pb intercept reached. In some cases the change in trend toward the origin is only defined by two or three data points (Lakes 224, 226SW, 240, and 259; Fig. 4D, F, I, and J). In other lakes (222, 223, 226NE, 227, 302N, and 302S) several points follow a straight line providing a well defined trend of the data toward the origin (Fig. 4B, C, E, G, K, and L). Some form of downward redistribution of ^{137}Cs must have occurred to produce this trend, and it has occurred in each of the lakes studied. Benthic organisms present in ELA lakes (Table 2) are incapable of significantly mixing sediments to depths greater than $\simeq$2 cm. Two questions arise then: (1) What is the nature of the redistribution process? (2) Is the redistribution limited to ^{137}Cs, or has it affected ^{210}Pb and the bulk sediments as well?

Sediment Focussing

Particle-reactive elements added in solution to the surface waters of a lake are removed to the sediments by two competing processes: direct adsorption to epilimnion sediments in

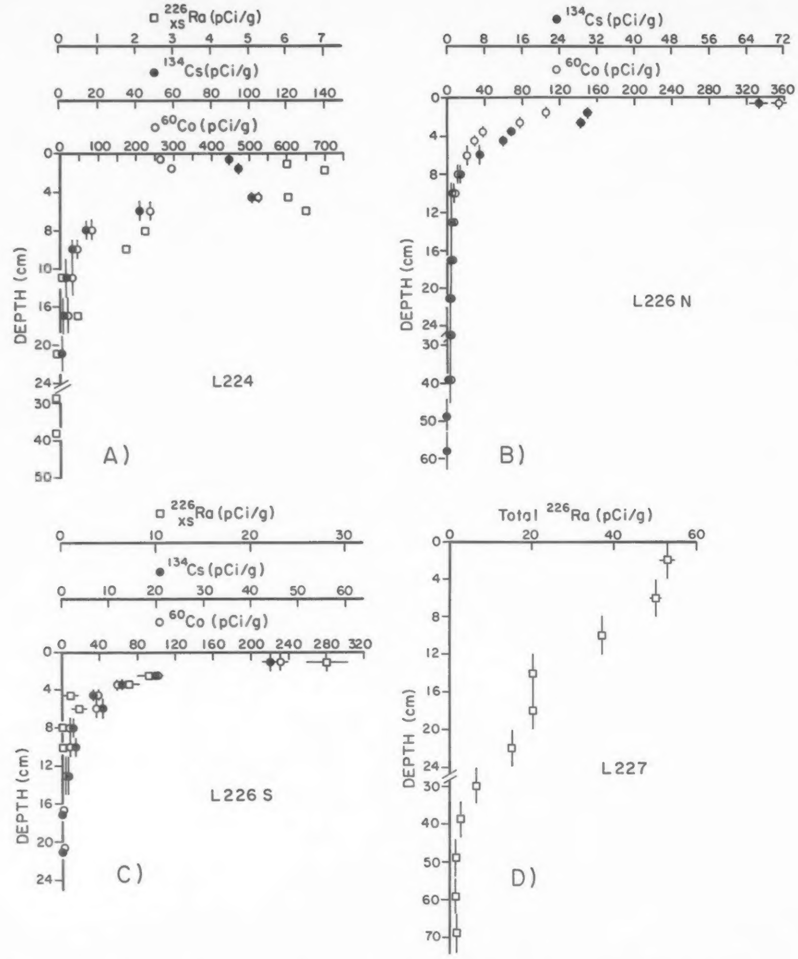


FIG. 5. Distributions of experimentally added radiotracers in the sediments of 4 ELA lake basins. A = L224, B = L226N, C = L226S, D = L227. ^{226}Ra was added to L227 in 1970, to L224 in 1976, and to L226 in 1978. ^{134}Cs and ^{60}Co were added to L224 in 1976 and to L226 in 1977.

contact with the water and adsorption to particulate matter present in the water column (e.g., Hesslein et al. 1980; Santschi et al. 1986). The relative importance of the two processes depends largely on the solubility of the element, with highly insoluble elements being primarily removed by adsorption to settling particles whereas elements of intermediate solubility are primarily removed by direct adsorption to surface sediments (see Santschi et al. 1986 for details applicable to ELA lakes). Lead is a highly insoluble element, so its removal to settling particles initially distributes ^{210}Pb in a relatively uniform manner over the lake bottom. Cesium has an intermediate solubility, and thus it is primarily removed by adsorption to littoral sediments (Hesslein et al. 1980; Santschi et al. 1986).

Fine-grained littoral sediments can be resuspended during the lake mixing events and transported to greater water depths. This winnowing process has been shown to occur in ELA Lake 227 (Emerson and Hesslein 1973; Emerson 1974). M. Davis and coworkers (e.g., Davis 1973; Likens and Davis 1975; Davis et al. 1984a) have shown that sediment focussing is a general phenomenon in lakes. More recently, the processes of sediment focussing have been modelled and reviewed by Hilton (1985) and by Hilton et al. (1986).

Sediment chronologies obtained using xs^{210}Pb results involve normalizing the xs^{210}Pb concentrations at depth to the surface concentration (see below). Therefore, if winnowing is effectively a constant process on a time scale of years and longer, then the activity of xs^{210}Pb in surface sediments at a depositional center for focussed sediment will remain constant with time and the xs^{210}Pb gradient in the sediments will give an accurate measure of the sediment accumulation rate provided that the other necessary assumptions (see below) are valid. Sediment focussing will not affect the first appearance of a transient tracer in the sediments at a depositional site. However, in the same sediments, the maximum concentration of a transient tracer initially incorporated into epilimnion sedi-

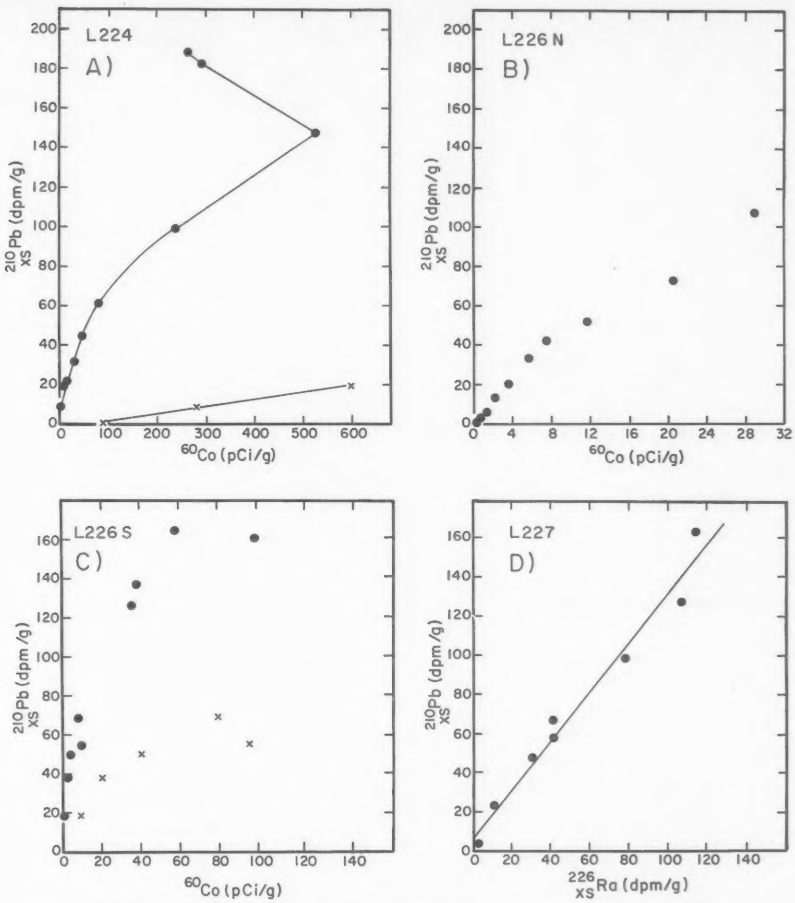


FIG. 6. Unsupported ^{210}Pb activities are plotted against ^{60}Co activities in L224 (A), L226N (B), and L226S (C) and against ^{226}Ra activities in excess of natural levels in L227 (D) to illustrate the linear relationship between the distribution of xs^{210}Pb and the distributions of these radionuclides in the deeper portions of these three cores. ^{60}Co results are replotted as "X"'s at 100 times the activity scale in (A) and at 10 times the activity scale in (C) to show that the ^{60}Co activities are significantly above zero in the deeper portions of these cores.

ments may post date its peak input to the lake by several years as the result of the winnowing process.

Inventories of ^{210}Pb in the cores (Table 1) were generally much greater than the value of 16 dpm·cm^{-2} expected from the quantitative burial of ^{210}Pb deposited from the atmosphere in this area (≃0.5 dpm·cm^{-2}·yr^{-1}; Brunskill and Wilkinson, 1987). Supply of ^{210}Pb from terrestrial runoff provides less than 10% of the total ^{210}Pb input to ELA lakes (Brunskill and Wilkinson 1987). Therefore, focussing of sediments from shallow areas to deeper portions of these lakes must occur to account for such high inventories. Accumulation rates of fine-grained sediments in the deepest parts of these lakes where the cores were collected must then be much greater than the lake-wide average accumulation rates.

^{137}Cs inventories (Table 1) ranged both above and below that expected from decay-corrected atmospheric fallout at this location of 78 mCi km^{-2} (calculated from the fallout record at International Falls, Minnesota). Winnowing of littoral sediments may have occurred to variable extents in these lakes, thereby accounting for the different ^{137}Cs inventories, and for the differernt ^{137}Cs/^{210}Pb inventory ratios, among these lakes. The relative importance of the two processes removing Cs and Pb from the water column may also vary from lake to lake, further contributing to the between-lake difference in ^{137}Cs/^{210}Pb inventory ratios.

Apparent Sedimentation Rates from ^{210}Pb and ^{137}Cs Distributions

Sedimentation rates calculated from the first appearance of ^{137}Cs (1953) and the peak in ^{137}Cs activity (1963) are compared in Table 3 with sedimentation rates calculated (Equation 2) from the log-linear portions of the xs^{210}Pb distributions. Sedimentation rates calculated from the deepest appearance of ^{137}Cs are much greater than rates obtained from xs^{210}Pb gradients, as expected from its deeper than expected occurrence.

Sedimentation rates based on the depth of the ^{137}Cs peak (or upper limits where peaks were not observed) are generally lower than rates based on ^{210}Pb results, contrary to the expected effect of sediment mixing or diffusion of Cs in pore waters. Mixing would cause the ^{137}Cs peak to be shifted downward (Robbins and Edgington 1975; Robbins et al. 1977; and see below), causing apparent sediment accumulation rates to be greater than rates derived from ^{210}Pb distributions. Winnowing of fine-grained sediments from littoral zones to greater depths could maintain a high rate of supply of ^{137}Cs to these deeper sites after 1963, thereby accounting for the upward displacement of the ^{137}Cs peaks.

Constraints on Sediment Accumulation Rates

Sediment accumulation rates calculated from the slope of the log-linear portions of the xs^{210}Pb profiles (Table 3) represent upper limits for the true sedimentation rates because any sediment mixing over the log-linear interval will decrease the xs^{210}Pb gradient, thereby increasing the apparent sedimentation rate calculated by applying Equation 4. Lower limits of the deposition rate of fine grained sediments over the whole lake area, r (g·cm^{-2}·yr^{-1}), which is related to S (cm·yr^{-1}) by $r = S(1 - \phi)\rho$, can be obtained by dividing the atmospheric ^{210}Pb deposition rate ($F = 0.5$ dpm·cm^{-2}·yr^{-1}) by the ^{210}Pb activity of newly sedimenting particles (A_s)

$$(7) \qquad r = F/A_s$$

Extrapolating measured ^{210}Pb distributions to the sediment–water interface suggests initial ^{210}Pb activities of 100 to 200 dpm·g^{-1}. Whole-lake mass accumulation rates would then be 0.0025–0.005 g·cm^{-2}·yr^{-1}. Sediment focussing to deeper portions of the lakes may increase the local accumulation rates of fine grained material by up to an order of magnitude (Table 3) above the whole-lake value.

Independent estimates for sediment accumulation rates are available for some of these lakes. G. Davidson (Freshwater Institute, personal communication, 1985) has collected cores from Lakes 223 and 227 by an in situ freezing technique which eliminates sediment disturbance during coring. He found a sedimentation rate based on the accumulation of visible annual layers in the sediments of 0.0021 g·cm^{-2}·yr^{-1} (0.95 mm·yr^{-1} in the upper few mm) in Lake 223, and a 3.0 cm accumulation of layers covering a 5.5 year interval in the surface sediments of Lake 227. An accumulation rate of 0.8 mm·yr^{-1} was calculated for a L223 core collected near the site of our core based on an increase in ambrosia pollen content at 9 cm (M. Dickman, unpublished results). A long core collected at 9 m water depth in L240 contained about 6 m of sediment above the laminated clays deposited at the time of the retreat of the glaciers in this area about 10 000 years ago (Stockner 1971). The average accumulation rate over this period of $\simeq$0.6 mm·yr^{-1} must be lower than the accumulation rate of surface sediments because of compaction. These independently derived accumulation rates are close to the values obtained from the xs^{210}Pb gradients (Table 3), suggesting that the ^{210}Pb-based rates are not in error on the high side due to sediment mixing by more than a factor of two.

Distributions of Experimentally Introduced Radionuclides

Experimentally added nuclides were measurable (at least 3 sigma above blank) to depths of at least 15–20 cm in each of the four spiked lakes (Fig. 5). In the absence of post depositional mobility the maximum depths of these radionuclides predicted from ^{210}Pb-based sedimentation rates would have been about 5 cm in L227 and about 2 cm in Lakes 224, 226N, and 226S. Distributions of experimentally added radiotracers show a relationship to xs^{210}Pb profiles (Fig. 6) similar to that observed for ^{137}Cs (Fig. 4). Data points from the deeper sections of cores from Lakes 224 and 226N fall tightly about a straight line (Fig. 6A and B). In L226N, this zone over which the xs^{210}Pb and ^{60}Co distributions are linearly related covers a depth interval between the 9–11 cm and 43–53 cm samples. The relationship between xs^{226}Ra and xs^{210}Pb in Lake 227 (Fig. 6D) is linear to depths $>$40 cm, with the trend passing through the origin. A plot of xs^{210}Pb against ^{60}Co in L226S (Fig. 6C) also shows downward curvature, although the data do not fall tightly about a straight line as for the other cores.

Penetration of ^{137}Cs, ^{60}Co, ^{134}Cs, and ^{226}Ra to much greater depths than predicted provides conclusive evidence for the downward transport of Cs, Co, and Ra in these sediments. The linear relationships between xs^{210}Pb and ^{137}Cs (Fig. 4), ^{60}Co (Fig. 6A, B), and ^{226}Ra (Fig. 6D) in the deeper parts of these cores suggests that these distributions may be controlled by some type of mixing process. In the following sections these profiles will be modelled to examine whether or not the measured distributions can be reproduced by a consistent set of accumulation, mixing, and diffusion rates.

Discussion

Two types of inconsistencies between the measured distributions of ^{137}Cs (also applicable to ^{134}Cs and ^{60}Co) and xs^{210}Pb were described in the preceding sections: (1) ^{137}Cs was found at much greater depths in the sediments than expected, and (2) expected peaks in the profiles of ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra either did not occur or were displaced upward from their expected depths based on xs^{210}Pb accumulation rates. Davis et al. (1984b) found ^{210}Pb and ^{137}Cs distributions in cores from a number of lakes in New England and in Norway which were similar to the distributions reported here for ELA lakes (Fig. 2) in that ^{137}Cs was observed to penetrate to much greater depths than would be expected from ^{210}Pb chronologies and maximum ^{137}Cs concentrations were displaced toward the sediment–water interface. The explanation offered by Davis et al. (1984b) was that Cs is more soluble than Pb, and consequently there was significant transport of ^{137}Cs dissolved in pore waters. They invoked a coincidence that ^{137}Cs penetrated to the same depth as xs^{210}Pb in most of their cores. The maximum concentration near the sediment–water interface was ascribed to upward diffusion followed by immobilization near the sediment–water interface by adsorption.

As noted above, processes other than diffusion in pore waters such as sediment focussing and sediment mixing may also affect ^{137}Cs profiles. Therefore, some numerical modelling exercises were carried out to determine if our measured profiles of ^{210}Pb, ^{137}Cs, ^{134}Cs, ^{60}Co and ^{226}Ra could be explained by common sets of parameters for sediment accumulation and mixing rates when effects of sediment winnowing and diffusion in pore waters are included.

Numerical Model

The advantages of numerical modelling are that parameters such as sedimentation rate, mixing rate, and porosity can be

TABLE 4. In situ distribution coefficients determined from a composite sediment sample over a depth interval of approximately 0–20 cm collected near the point of maximum depth in Lake 226NE.

Isotope	Sediment (pCi/kg)	Pore water activity (pCi/kg)	K_D[a]
^{134}Cs	4327 ± 93	1.33 ± 0.33	3.25 ± 0.81 × 10^3
^{137}Cs	9508 ± 166	1.83 ± 0.36	5.2 ± 1.0 × 10^3
^{60}Co	60052 ± 628	52.53 ± 0.87	1.14 ± 0.02 × 10^3
^{210}Pb	45909 ± 909	1.05 ± 0.03	4.37 ± 0.14 × 10^4
^{226}Ra (^{214}Bi)	1231 ± 116	1.1 ± 0.8	1.2 ± 0.9 × 10^3

[a] K_D is defined as [activity/g (dry sediment)]/[activity/g (pore water)].

varied with depth, and variable input rates such as global fallout from nuclear weapons testing can be incorporated into the model. We have used a multiple box sediment mixing model which incorporates numerical solutions to Equation (2) (Peng et al. 1979). Earlier versions of the model have been described by Peng et al. (1979), Santschi et al. (1980), and Olsen et al. (1981). The sediment column is divided into boxes of thickness Δz, and further divided into two layers $0 < z < L$ and $z > L$. Sediment mixing rates could be either held constant, or could be decreased exponentially with depth, within each layer. Porosities were modelled as containing a constant term and an exponentially decreasing increment

$$(8) \qquad \phi(z) = \phi_{\mathrm{inf}} + \phi_{\mathrm{inc}} \cdot \exp(-A \cdot z)$$

where A is an empirical attenuation coefficient.

Sediment deposition rates, r (g·cm^{-2}·yr^{-1}), and radionuclide deposition rates, F (activity·cm^{-2}·yr^{-1}), were independent input variables. Sedimentation rate is a constant while F can be assigned any input function (e.g. the ^{137}Cs fallout record). The fallout record of ^{137}Cs at ELA was estimated from the measured ^{90}Sr deposition record at International Falls, Minnesota (Hardy 1977; EML 1982), the closest monitoring station to ELA. Data from International Falls were not available for years prior to 1959, so fallout records from New York City prior to 1959 were used after multiplying by a factor of 0.71, the ratio of post 1958 integrated fallout at International Falls to the integrated fallout at New York City (EML 1982). The ^{90}Sr records were then multiplied by 1.5, the average ^{137}Cs/^{90}Sr ratio in fallout, to arrive at the ^{137}Cs deposition record for ELA.

Input of the experimentally added nuclides was modelled either as a pulse having a duration of 0.1 yr, or as being continuous after the time that the lake was spiked, i.e., the winnowing effect. Supply of ^{137}Cs to the sediments under the winnowing effect was modelled as being proportional to the fallout record until 1963, and thereafter the 1963 fallout levels were maintained.

Sediment and radionuclide deposition, sediment mixing between boxes, and radioactive decay were carried out in incremental time steps (Δt). Typical values for Δt and Δz were 2 d and 0.2 cm, respectively. Comparisons between model runs using these values and runs using values of 1 d and 0.1 cm showed insignificant differences. Model simulations covered 200 yr for ^{210}Pb to achieve steady state, 30 yr for ^{137}Cs (1950–80), and for the number of years that had elapsed between experimental addition of radiotracers and coring (3–10).

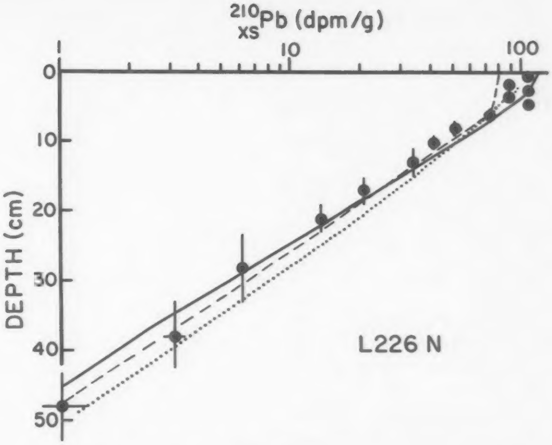


FIG. 7. Model-generated profiles of xs^{210}Pb are compared with the measured distribution of xs^{210}Pb in L226N. Dashed curve is the fit for sediment accumulation at 0.0225 g·cm^{-2}·yr^{-1} and sediment mixing in the upper 5 cm at 1.5 × 10^{-7} cm^{-2}·s^{-1}. Conditions for the dotted line are the same as for the dashed line except that diffusion of ^{210}Pb in pore waters at a D_{eff} of 1.4 × 10^{-9} cm^2·s^{-1} is included. The solid line is the model prediction for negligible sediment accumulation where the ^{210}Pb distribution is determined entirely by sediment mixing. The sediment mixing rate is 1.5 × 10^{-7} cm^2·s^{-1} above 5 cm and 7.3 × 10^{-8} cm^2·s^{-1} below 5 cm.

Rates of nuclide transport by diffusion in pore waters depend on the free ion diffusion coefficient, D_m, the porosity ϕ, and the distribution coefficient, K_D, for partitioning of the nuclide between solution and the solid phase (Lerman 1979). Effective diffusion coefficients (D_{eff}) for the transport of nuclides by diffusion in pore waters were calculated (Lerman 1979) as

$$(9) \qquad D_{\mathrm{eff}} = \frac{D_m \Phi^2}{[1 + K_D(1-\Phi)\rho_s/\Phi]}$$

Distribution coefficients (K_D) were calculated from the measured concentrations of the nuclides in L226N pore waters and associated solids (Table 4). Values of D_m used for Cs, Co, Ra, and Pb were 12 × 10^{-6}, 4 × 10^{-6}, 5 × 10^{-6}, and 5 × 10^{-6} cm^2·s^{-1} (≃4°C), respectively (Lerman 1979; Li and Gregory 1974). At $\phi = 0.97$, typical for most of these lakes, D_{eff} for Co and Cs differ by less than 20% because the larger K_D of Cs is nearly compensated by its larger D_m. Separate models were therefore not run for both Cs and Co.

Unsupported ^{210}Pb gradients in the sediments depend on the mass accumulation rate, the porosity, and on the combined rate of sediment mixing and diffusion in pore waters. A test of the mixing model is that it should reproduce the observed xs^{210}Pb distribution given all of the input parameters. Using the measured porosity and the mass accumulation rate calculated from the xs^{210}Pb gradient in L226N as input parameters, and including sediment mixing to 5 cm, the model accurately reproduced the measured xs^{210}Pb distribution (Fig. 7, dashed line). Pb is much less soluble than the other nuclides measured in this study (Table 4), and consequently its distribution should be the least affected by diffusion in pore waters. This is illustrated by the dotted line in Fig. 7 which shows that including the small

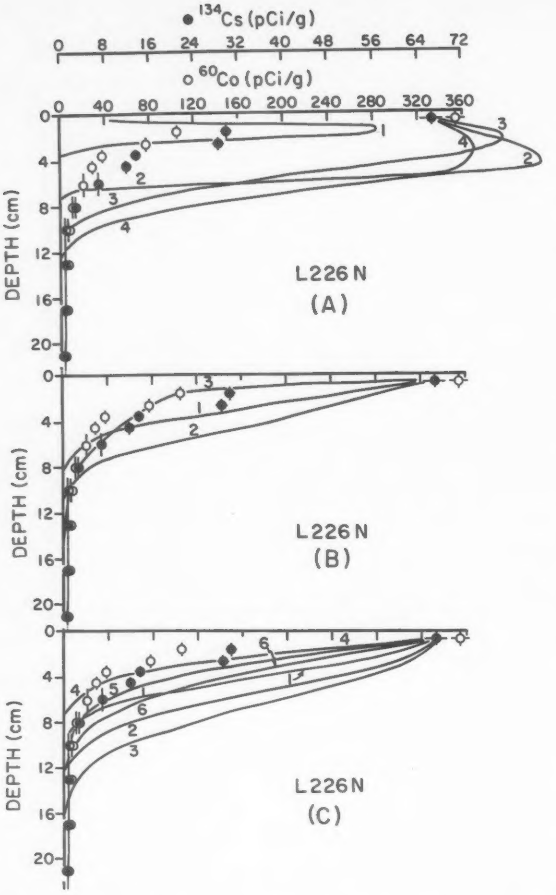


FIG. 8. Predicted distributions of ^{134}Cs and ^{60}Co for different rates of sediment mixing and of diffusion in pore waters are compared with the measured ^{134}Cs and ^{60}Co distributions in L226NE; $r = 0.0225\ \mathrm{g \cdot cm^{-2} \cdot yr^{-1}}$ in A and B, $r = 0$ in C, $\phi(z) = 0.970 + 0.015 \exp(-0.2\,z)$ for all curves. (A) Pulse input situation (see text): Curve 1, no sediment mixing or diffusion, predicted concentrations are reduced to keep the profile on scale. Curve 2: sediment mixing to 5 cm at $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$, $D_{\mathrm{eff}} = 0$. Curve 3: $D_B = 0$, $D_{\mathrm{eff}} = 7.5 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at $z = 0$ to $3.2 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at the bottom. Curve 4: $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ to 5 cm, D_{eff} as in curve 3; (B) Continuous input situation (see text). Curve 1: D_B and D_{eff} same as in curve 3 above. Curve 2: D_B and D_{eff} same as in curve 4 above. Curve 3: $D_B = 0$, $D_{\mathrm{eff}} = 0$ to 0.6 cm, D_{eff} decreases exponentially from $2.9 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ at 0.6 cm to $1.7 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ at the bottom; (C) Model predictions for conditions of negligible sediment accumulation, $r = 0$. Curves 1, 2, and 3 for pulse input and Curves 4, 5, and 6 for continuous input. Curves 1 and 4, $D_B = 0$, D_{eff} as in Curve 3 of A. Curves 2 and 5, D_B and D_{eff} same as in curve 3 of A. Curves 3 and 6: D_B and D_{eff} same as in curve 4 of A.

effective diffusivity for ^{210}Pb ($1.4 \times 10^{-9}\ \mathrm{cm^2 \cdot s^{-1}}$ at $\phi = 0.97$) calculated from its K_D (Table 4 and Equation 6) had little effect on the xs^{210}Pb gradient. These input parameters were therefore used to model the distribution of other radionuclides in L226N. This lake was chosen for the most intensive modelling because it provided the best, and most complete, set of results for ^{210}Pb, ^{137}Cs, and experimentally added ^{134}Cs and ^{60}Co.

Three situations were modelled. Input functions used for the pulse and continuous (winnowing) supply situations were described above. These situations were both modelled using sediment accumulation at the rate calculated from the xs^{210}Pb gradient (Table 3; $0.022\ \mathrm{g \cdot cm^{-2} \cdot yr^{-1}}$ for L226N). Sediment accumulation was assumed to be negligible in the third situation, and the entire downward distribution of xs^{210}Pb was assumed to result from sediment mixing. Sediment mixing rates calculated from the xs^{210}Pb gradients (equation 6) were applied to the other nuclides in this case.

Pulse Input Situation

Model runs to simulate pulse input conditions for L226NE produced distinct subsurface peaks in the profiles of ^{137}Cs, ^{134}Cs, and ^{60}Co, whether or not sediment mixing or diffusion in pore waters was included. In the absence of diffusion or mixing, the distributions of ^{134} Cs and ^{60}Co would have exhibited a sharp maximum at 2 cm (Fig. 8A, curve 1). There would have been a sharp peak in the ^{137}Cs distribution corresponding to 1963 at 6–7 cm (Fig. 9A, curve 1). Including a 5-cm mixed layer evident in the ^{210}Pb profile (Fig. 7) shifted the ^{134}Cs–^{60}Co peak down to 4–5 cm (Fig. 8A, curve 2) and shifted the ^{137}Cs peak down to about 9 cm (Fig. 9A, curve 2). This illustrates a general effect of mixing in the upper part of the sediment column. Peak distributions of transient tracers, whether nuclides, pollen, etc., are displaced downward from the position of the sediment–water interface at the time of the event.

Effective diffusion coefficients (D_{eff}) and sediment mixing coefficients (D_B) were assumed to be additive, and were combined into a single parameter ($D_S = D_{\mathrm{eff}} + D_B$) for downward nuclide transport. Allowing nuclides to diffuse in pore waters according to their D_{eff} values caused the ^{134}Cs–^{60}Co peak to be slightly shallower (2–3 cm without mixing, Fig. 8A, curve 3; 3–4 cm with mixing to 5 cm, curve 4) than for the case of sediment mixing to 5 cm without diffusion in pore waters (Fig. 8A, curve 2). The ^{137}Cs peak remained at about 9 cm, both with and without sediment mixing to 5 cm, when pore water diffusion was included (Fig. 9A, curves 3 and 4).

Without diffusion in pore waters ^{137}Cs would have been undetectable below 16 cm even with sediment mixing in the upper 5 cm (Fig. 9A, curve 2). However, including diffusion in pore waters transported measurable amounts of ^{137}Cs down to 25–30 cm (Fig. 9A, curves 3 and 4).

Pulse-input model predictions are clearly inconsistent with the observed distributions of ^{134}Cs, ^{60}Co, and ^{137}Cs in L226N where the predicted peaks were not found for any of the nuclides. Peaks in the distributions of ^{134}Cs and ^{60}Co were similarly predicted for Lakes 224 and 226S (model curves not shown). The loss of critical samples between 2–4 cm in L224 precludes distinguishing between a major peak in this region and a uniform mixed layer to ≃5 cm (Fig. 5A). Lake 226S appears to be similar to L226N in that ^{134}Cs and ^{60}Co concentrations decrease sharply downward from the surface (Fig. 5C). ^{137}Cs peaks were similarly absent, or occurred at shallower depths than expected in Lakes 222, 223, 227, 240, 259, 302N, and 302S (Fig. 2). The absence of the predicted peaks probably results from the continued input of the nuclides by winnowing from shallower sediments (see above).

Winnowing does not affect the distribution of ^{137}Cs equally in all lakes. For example, the distribution of ^{137}Cs in L239 was modelled using values of porosity and sedimentation rate mea-

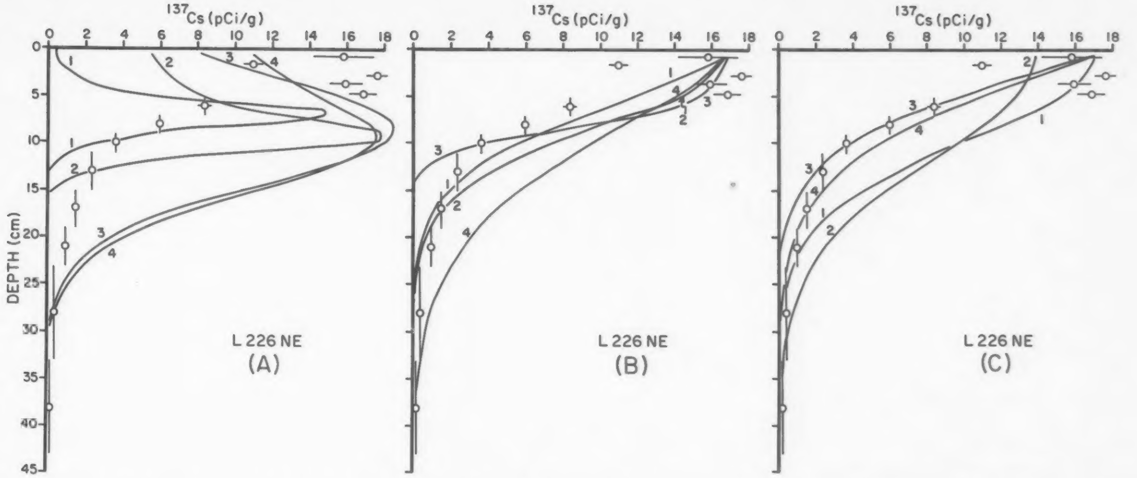


FIG. 9. Predicted distributions of ^{137}Cs for different rates of sediment mixing and of diffusion in pore waters are compared with the measured ^{137}Cs distribution in L226NE; $r = 0.0225\ \mathrm{g \cdot cm^{-2} \cdot yr^{-1}}$ in A and B, $r = 0$ in C, $\phi(z) = 0.970 + 0.015 \exp(-0.2\,z)$ for all curves. (A) Pulse input situation (see text): Curve 1, no sediment mixing or diffusion, predicted concentrations are reduced to keep the profile on scale. Curve 2: sediment mixing to 5 cm at $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$, $D_{eff} = 0$. Curve 3: $D_B = 0$, D_{eff} decreases exponentially from $7.5 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at $z = 0$ to $3.2 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at the bottom. Curve 4: $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ to 5 cm, D_{eff} as in curve 3; (B) Continuous input situation (see text). Curve 1: $D_B = 0$, D_{eff} same as in curve 3 above. Curve 2: D_B and D_{eff} same as in curve 4 above. Curve 3: $D_{eff} = 0$, $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ to 5 cm. Curve 4. $D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ to 5 cm, D_{eff} decreases exponentially from $2.9 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ at the sediment−water interface to $1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ at the bottom ($K_D = 1000$); (C) Model predictions for conditions of negligible sediment accumulation, $r = 0$. Curves 1, and 2 for pulse input and Curves 3 and 4 for continuous input. Curves 1 and 3, $D_B = 0$, D_{eff} as in Curve 3 of A. Curves 2 and 4, D_B and D_{eff} same as in curve 4 of A.

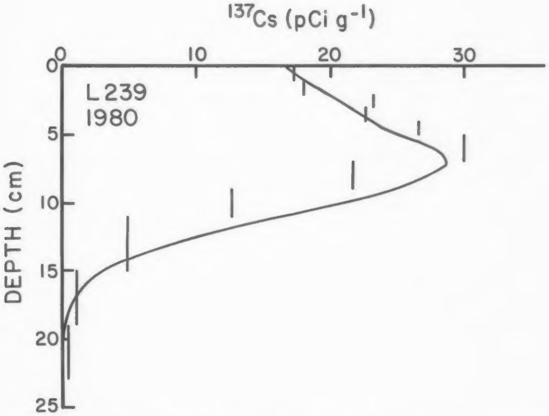


FIG. 10. Model predicted ^{137}Cs distribution in L239 is compared with the measured distribution using the fallout record as the ^{137}Cs input function. Model parameters: $r = 0.028\ \mathrm{g \cdot cm^{-2} \cdot yr^{-1}}$, $\phi = 0.90 + 0.08 \exp(-0.1\,z)$, $D_{eff} + D_B = 1.5 \times 10^{-7}\ \mathrm{cm^2 \cdot s^{-1}}$ above 5 cm. Below 5 cm $D_B = 0$, and D_{eff} decreases exponentially from $2.1 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at 5 cm to $1.1 \times 10^{-8}\ \mathrm{cm^2 \cdot s^{-1}}$ at the bottom.

sured in L239 and the Cs K_D value measured in L226N. The peak in the ^{137}Cs distribution is fairly well reproduced (Fig. 10), indicating a minimal delay in ^{137}Cs supply by winnowing. There does not appear to be a consistent relationship between the magnitude of the winnowing effect and observable lake features such as area or water depth.

Continuous Input Situation

Allowing a continuous supply of nuclides to the sediments eliminated the peaks from the predicted nuclide distributions (Fig. 8B and 9B). Measurable amounts of ^{134}Cs and ^{60}Co could have penetrated to 10 cm under the combined effects of sediment mixing to 5 cm and pore water diffusion at all depths (Fig. 8B, curve 2). However, curve 2 does not reproduce the sharp concentration gradient observed in the upper 0−3 cm. Eliminating sediment mixing increased the predicted concentration gradient in the upper 3 cm (Fig. 8B, curve 1), but decreased the depth to which the nuclides penetrated. Measurable amounts of ^{134}Cs and ^{60}Co were found much deeper than 10 cm, suggesting that the D_{eff} used to generate curves 1 and 2 in Fig. 8B was, if anything, too low. The observed steep ^{134}Cs and ^{60}Co gradients in the upper 3 cm and the deep penetration of these nuclides could only be reproduced if no diffusion or mixing at all was allowed in the surface few millimetres of sediments, and if D_{eff} below this depth was increased well above that expected from D_m and the measured values of ϕ and K_D (Fig. 8B, curve 3).

Immobilization of the nuclides near the sediment−water interface might not be entirely unreasonable if a thin layer of iron oxides, or some other authigenic solid phase which had a very high binding affinity for the nuclides, existed in the surface sediments. Deeper, more reducing sediments would be free of the Fe oxides, and the D_{eff} of the nuclides deeper in the sediments would be much larger than near the sediment−water interface. Measuring the K_D using a 0−20 cm composite sample, as was done here, would not have detected any variability with depth in K_D.

Higher K_D values (very small D_{eff} in the upper few mm of sediments needed to account for the observed ^{134}Cs and ^{60}Co distributions are inconsistent with the ^{210}Pb and ^{137}Cs profiles. Nearly uniform concentrations of ^{210}Pb and ^{137}Cs to 5 cm in L226N (Fig. 7 and 9) required a lower limit for D_s in the upper 3 cm of sediments of $\simeq 1.5 \times 10^{-7}$ cm^2·s^{-1}. A conservative upper limit for sediment mixing in the upper 3 cm of L226N sediments permitted by the ^{134}Cs and ^{60}Co gradients was $\simeq 7 \times 10^{-9}$ cm^2·s^{-1}, about a factor of 20 lower than the lower limit derived from the ^{137}Cs and ^{210}Pb distributions. Modelling a continuous supply of ^{137}Cs did not produce a uniform 5-cm mixed layer (Fig. 9B), suggesting that the lower limit for the sediment mixing coefficient, 7.5×10^{-7} cm^2·s^{-1}, which was used in these model runs, may have been underestimated. Alternatively, the supply of ^{137}Cs may have decreased during recent years, which would also have had the effect of decreasing the gradient in the upper 5 cm.

The sharp gradient in ^{137}Cs concentration between 5–8 cm (Fig. 9B) limits D_s to less than $\simeq 5 \times 10^{-9}$ cm^2·s^{-1} in this region. This is inconsistent with a D_{eff} of 4.4×10^{-8} cm^2·s^{-1} calculated from the measured K_D of Cs (Table 4) at $\phi = 0.975$ (ϕ at 5–8 cm) and it is inconsistent with the much larger D_s required to distribute ^{137}Cs to depths greater than 30 cm (see below). An upper limit for D_s of 5×10^{-9} cm^2·s^{-1} is also inconsistent with the large D_s required to account for the ^{134}Cs profile over this depth interval. A D_s of $\simeq 2 \times 10^{-7}$ cm^2·s^{-1} in the 3–10 cm interval used to generate curve 3 in Fig. 8B actually overestimated the measured ^{134}Cs gradient in this region, so 2×10^{-7} cm^2·s^{-1} is a lower limit for ^{134}Cs migration. The inconsistency between mixing rates estimated from distributions of ^{134}Cs and ^{137}Cs was greater than a factor of 20 in the 5–8 cm region, but in the opposite sense of the inconsistency of similar magnitude found in the 0–3 cm region.

The requirement that D_s increases with depth was not unique to L226N. It was the rule, rather than the exception. It was required to explain the measured distributions of ^{134}Cs and ^{60}Co in Lakes 224 and 226S (Fig. 5A and C), as well as the distributions of ^{137}Cs in most of the lakes (Fig. 2).

Measurable levels of ^{137}Cs could have been transported to $\simeq$26 cm if diffusion in pore waters at the D_{eff} calculated from the average of the K_D values for ^{134}Cs and ^{137}Cs in Table 4 was allowed. However, this is still much less than the measured maximum depth of ^{137}Cs (>33 cm and possible >43 cm; Fig. 2E). The measured depth of ^{137}Cs penetration could only be reached through diffusion in pore waters if D_{eff} was increased by lowering K_D from 4000 to 1000 (Fig. 9B, curve 4). However, the predicted profile using such a high D_{eff} poorly reproduces the remainder of the ^{137}Cs distribution in L226N. The measured distribution of ^{137}Cs appears to require that D_s was high between 0–5 cm, low between 5–8 cm, and then increased again below 8 cm.

Nuclide Distributions Under Conditions of Negligible Sediment Accumulation

Model-generated radionuclide profiles for conditions of negligible sediment accumulation reproduced the observed nuclide distributions just as well as did the model-generated profiles for conditions where sediment accumulation occurred at the rate determined from the xs^{210}Pb gradient. It seems unlikely that the sediment accumulation rate is much less than the rates inferred from the xs^{210}Pb gradients because these rates are in good agreement with accumulation rates derived independently (see above). Annual laminations observed in Lakes 223 and 227 (Davidson, personal communication) argue against sediment mixing. Organisms capable of mixing sediments to depths of 10's of cm have not been observed in these lakes (Table 2). Mixing of these high-porosity sediments by methane bubble ebullition (Kelly and Chynoweth 1979), or other physical mixing processes may occur, but we know of no reported measurements from which mixing rates in lake sediments by these processes could be estimated. However, the topic is discussed briefly to demonstrate that nuclide distributions such as those in Fig. 1–5 could have been produced entirely by sediment mixing to great depths.

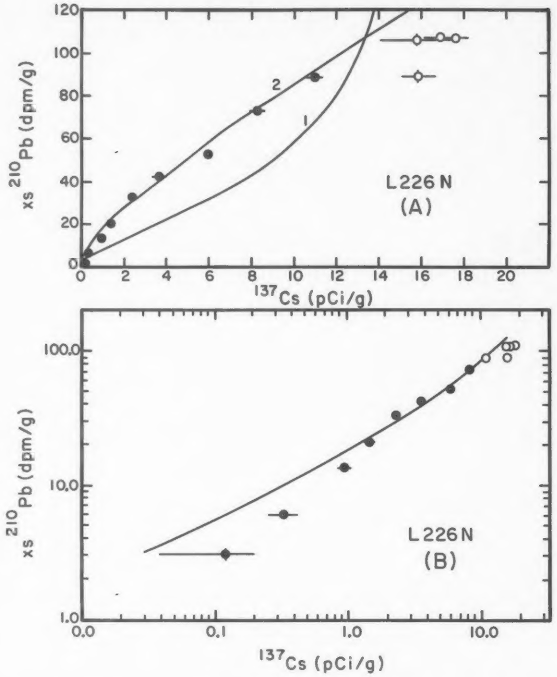


FIG. 11. Model-generated xs^{210}Pb–^{137}Cs relationships for conditions of negligible sediment accumulation are compared to the results from L226N. The xs^{210}Pb profile used to generate both curves in (A) is shown in Fig. 7 as a solid curve. Curve 1 is for the pulse input situation and uses the ^{137}Cs profile in Fig. 9C, curve 2. Curve 2 is for the continuous input situation and uses the ^{137}Cs profile in Fig. 9C, curve 4. Curve 2 from (A) is replotted on a log–log scale in (B) to show the deviation of the model curve from the data in the deeper part of the core.

Sediment mixing coefficients were set to reproduce the entire xs^{210}Pb distribution (Fig. 7, solid curve). Distributions of ^{134}Cs, ^{60}Co, and ^{137}Cs were then predicted using these mixing rates with, or without, enhancement of mobility by diffusion in pore waters. Distributions predicted using the pulse input functions did not reproduce the measured profiles of ^{134}Cs and ^{60}Co (Fig. 8C, curves 2 and 3) or of ^{137}Cs (Fig. 9C, curves 1 and 2). Better fits to the data were obtained using the continuous input model (Fig. 8C, curves 5 and 6; Fig. 9C, curves 3 and 4). Penetration of ^{134}Cs and ^{60}Co to more than 12 cm (Fig. 8C, curve 6) and penetration of ^{137}Cs to more than 30 cm (Fig. 9C, curve 4) could be obtained if the com-

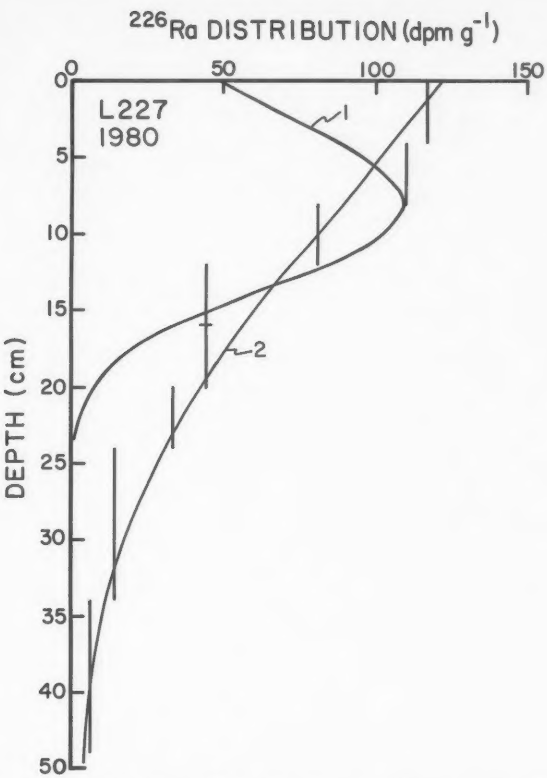


FIG. 12. Model predicted ^{226}Ra distributions in L227 are compared to the measured profile. Model parameters: $r = 0.021\ \mathrm{g\cdot cm^{-2}\cdot yr^{-1}}$, $\phi = 0.975 + 0.015\exp(-0.1z)$. Curve 1: Pulse input in 1970 and D_{eff} decreases exponentially from $1.5 \times 10^{-7}\ \mathrm{cm^2\cdot s^{-1}}$ at the surface to $6 \times 10^{-8}\ \mathrm{cm^2\cdot s^{-1}}$ at the bottom of the core, corresponding to the K_D for ^{226}Ra in Table 4. Curve 2: Continuous input since 1970, D_{eff} decreases exponentially from $1.6 \times 10^{-6}\ \mathrm{cm^2\cdot s^{-1}}$ at the surface to $0.5 \times 10^{-6}\ \mathrm{cm^2\cdot s^{-1}}$ at the bottom, corresponding to a K_D of 100. Model-generated curves are in arbitrary units and are scaled to correspond to the measured activities.

bined effect of sediment mixing and diffusion in pore waters was allowed.

The continuous input model reproduced most of the linear xs^{210}Pb$-^{137}$Cs relationship in L226N when the xs^{210}Pb distribution was produced by sediment mixing alone and the ^{137}Cs distribution resulted from the combined effect of sediment mixing and pore water diffusion (Fig. 11A, curve 2). The pulse input model did not reproduce the type of xs^{210}Pb$-^{137}$Cs relationship observed in these lakes (Fig. 11A, curve 1). The modelled xs^{210}Pb$-^{137}$Cs relationship for continuous ^{137}Cs input diverged from the data deeper in the core, as shown more clearly in Fig. 11B. The divergence is in the direction that would require an increase with depth in the rate of downward ^{137}Cs transport. This is similar to the situation described in the preceding section where nearly all of the measured distributions of ^{134}Cs, ^{60}Co, and ^{137}Cs required a D_s that increases with depth over some depth interval. It was, in fact, the sharp gradients in ^{134}Cs and ^{60}Co between 0$-$3 cm, and the ^{137}Cs gradient between 5$-$8 cm, which were used in the preceding section to identify the need for a D_s that increased with depth, that the Negligible Sediment Accumulation model was least able to reproduce. All of the models appear to require the unexpected situation that D_s increases with depth in the sediments of these lakes.

^{226}Ra Distributions

Distributions of xs^{226}Ra were generally similar to ^{134}Cs and ^{60}Co in Lakes 224 and 226S (Fig. 5), so they were not included in the preceding discussion. The distribution of ^{226}Ra in L227 provided a good illustration of the difficulty encountered in deriving sediment chronologies from the profiles of transient tracers for which the input function is not accurately known and for which diffusion in sediments can be significant. The distribution of ^{226}Ra in L227 sediments predicted for a pulse input corresponding to its injection into the lake in 1970, allowing for diffusion in pore waters in accord with its measured K_D (Table 4), is shown in Fig. 12 curve 1. A pronounced peak at 9 cm is predicted and the predicted maximum depth of penetration of $\simeq$23 cm is about half the depth to which measurable levels were found. Alternatively, modelling a continuous supply of ^{226}Ra after 1970 using a smaller value for K_D, but not outside the experimental uncertainty in the measured K_D value (Table 4), provided a good fit to the measured xs^{226}Ra distribution (Fig. 12, curve 2). These very different profiles (curves 1 and 2) illustrate the large range of ^{226}Ra distributions that would be possible given the uncertainty in the input function and in the value of K_D. Uncertainty in the input function and the K_D value are both critical factors which may preclude using the distributions of transient tracers to provide reliable chronologies in high-porosity sediments such as these.

Alternative Use of ^{210}Pb Results

If sediment mixing occurs to great depths, then the xs^{210}Pb gradients do not provide accurate sedimentation rates. However, some constraints may still be placed on the sedimentation rate. Sedimentation rates based on ^{210}Pb distributions would be upper limits for the true rates as any sediment mixing, diffusion, or downward smearing that affected the entire core would decrease the ^{210}Pb concentration gradient in the sediments.

An approach was described above to determine whole lake average sediment accumulation rates from the atmospheric flux of ^{210}Pb and the initial xs^{210}Pb activity of sedimenting particles (A_s). An alternative approach can be used to determine the sedimentation rate at an individual coring site. The sediment mass accumulation rate, r ($\mathrm{g\cdot cm^{-2}\cdot yr^{-1}}$) can be calculated from the xs^{210}Pb inventory, I ($\mathrm{dpm\cdot cm^{-2}}$) as

$$(10) \qquad r = \lambda I / A_s$$

where

$$(11) \qquad I = \int_0^\infty A(z)\,(1 - \phi)\,\rho_s\,dz$$

$A(z)$ is the xs^{210}Pb activity measured at each depth, z, and ρ_s is the dry density of the sediments. A problem arises in estimating A_s. Even if there is no mixing of the surface sediments, the ability to estimate the activity at the sediment$-$water interface by extrapolating from the measured activity profile is limited by the offset caused by the finite sampling intervals.

Where surface sediments are mixed by natural physical or biological processes, newly sedimented ^{210}Pb-rich particles are transported downward and replaced by older sediments which have lost some of their ^{210}Pb by decay, and the ^{210}Pb activity at the interface, $A(0)$, is less than A_s. Where sediment mixing is rapid enough to cause a uniform xs^{210}Pb activity in the mixed layer (e.g. Lakes 114, 224, 226NE, 239, 240, 259, 302N, and 302S), the relationship between $A(0)$ and A_s can be described by

$$\frac{A(0)}{A_s} = \frac{(S/L)}{(S/L) + \lambda} \tag{12}$$

where S is the sedimentation rate (cm·yr^{-1}) and L is the mixed layer depth (cm). If S and L are converted to units of g·cm^{-2}·yr^{-1} (r) and g·cm^{-2} (L'), then the equation becomes

$$\frac{A(0)}{A_s} = \frac{(r/L')}{(r/L') + \lambda} \tag{13}$$

Substituting for A_s in Equation (10) gives

$$\frac{A(0)\cdot r}{\lambda\cdot I} = \frac{(r/L')}{(r/L') + \lambda} \tag{14}$$

Combining like terms and solving for r gives

$$r = (\lambda I/A(0) - \lambda L') \tag{15}$$

Thus sedimentation rates can be calculated for these cores independently of the log-linear xs^{210}Pb distribution. Sedimentation rates calculated using Equation (15) are included in Table 3. Sedimentation rates calculated using this approach are not much less than the rates calculated from the xs^{210}Pb gradients over the log-linear portions of the distributions. Although the ^{210}Pb distributions may have suffered slightly from downward mixing or smearing, the agreement between columns 3 and 4 in Table 3 indicates that any error in the sedimentation rate derived from the xs^{210}Pb gradient is much less than a factor of two. Better porosity data would be required for more quantitative comparisons.

Summary and Conclusions

Measured profiles of xs^{210}Pb, ^{137}Cs, ^{134}Cs, ^{60}Co and ^{226}Ra in the sediments of ELA lakes required sediment accumulation and/or mixing rates that were internally inconsistent based on the known histories of supply of these nuclides to the lakes. These inconsistencies include:

1) ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra were found much deeper in the sediments than expected based on their known input histories and xs^{210}Pb-derived sedimentation rates. In every lake, measurable amounts of ^{137}Cs (and ^{134}Cs, ^{60}Co, and ^{226}Ra in the lakes to which they were added) were found at the maximum depth of detectable xs^{210}Pb. In the deeper parts of most of these cores, highly correlated linear relationships between the distributions of xs^{210}Pb and of ^{137}Cs (as well as between ^{210}Pb and ^{60}Co or ^{226}Ra) were found.

2) Subsurface peaks in the sediment profiles of ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra corresponding to the times of maximum nuclide supply to the lakes either were absent, or were displaced upward from the expected depths of the peaks based on xs^{210}Pb chronologies.

3) Sediment profiles of ^{137}Cs, ^{134}Cs, and ^{60}Co required situations for which the rate of vertical (downward) transport in the sediment column, whether by sediment mixing or by diffusion in pore waters, increased with depth in the sediments.

4) Rates of vertical migration of Cs in the sediments determined independently from the coexisting distributions of ^{137}Cs and ^{134}Cs were highly discordant, differing by more than a factor of 20 over two different depth intervals in the L226N core.

Some of the above inconsistencies have satisfactory explanations. The absence, or upward displacement, of peaks in the profiles of ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra probably resulted from the continued supply of these nuclides to the coring sites by winnowing of sediments from shallower depths where most of the nuclide removal from the water column initially occurred. Inventories of xs^{210}Pb in the cores much greater than could have been supported by atmospheric deposition of ^{210}Pb also indicated the importance of winnowing and sediment focussing in these lakes. Redistribution of nuclides in the lakes involved downward migration within the sediment column as well as lateral transport by winnowing and sediment focussing. Downward transport occurred both by sediment mixing, which was evident in the upper few cm of some of the ^{210}Pb profiles, and by diffusion in pore waters. Sediment mixing and diffusion may account, in part, for the occurrence of ^{137}Cs, ^{134}Cs, ^{60}Co, and ^{226}Ra deeper than expected in these cores.

Penetration of ^{137}Cs to the same depth as xs^{210}Pb, and a linear relationship between ^{137}Cs and xs^{210}Pb, might be explained as a coincidence if it occurred only once. However, it was observed in each of the ELA lakes cored, even though the depth of maximum nuclide penetration varied among the lakes between 10 and >50 cm. It seems highly improbable that exactly the necessary, but different, combination of accumulation rate, porosity, and K_D existed in each of these lakes to cause ^{137}Cs to penetrate by diffusion in pore waters to exactly the maximum depth of measurable xs^{210}Pb. This feature is perhaps the strongest evidence that the nuclide distributions have been affected by some form of sediment mixing, whether natural or an artifact of the coring procedure. The linear xs^{210}Pb–xs^{226}Ra relationship over the entire 40-cm sediment profile in L227 is also suggestive of sediment mixing, although the possibility that the ^{226}Ra profile was produced by diffusion of Ra in pore waters cannot be ruled out.

Penetration of ^{134}Cs and ^{60}Co to the maximum depth of detectable xs^{210}Pb activity is a strong argument for some form of rapid sediment mixing, or perhaps for downward smearing of surface sediments during core collection or extrusion. Because the time available for diffusion of ^{134}Cs and ^{60}Co in pore waters was much less than for ^{137}Cs, it is unlikely that measurable concentrations of the three nuclides would have penetrated to the same depth by diffusion. Downward mixing or smearing during sampling is a more reasonable explanation. The extent to which the xs^{210}Pb distributions, and consequently the xs^{210}Pb-derived sedimentation rates, would have been affected by this artifact would depend on the nature of the process. If it involved the downward transport of a small amount of surface sediments, then the effect on the xs^{210}Pb distribution may have been small. Alternatively, if the process involved mixing that only began after the core had penetrated several cm into the sediments, then the xs^{210}Pb distributions may have been severely affected. The nature of the coring artifact, if it occurred at all, cannot be determined from the available data. Additional tests of the sampling procedure will be required to fully evaluate the nature and extent of this possible coring artifact.

A coring artifact involving downward smearing of the sediments cannot explain all of the inconsistencies noted at the beginning of this section. Specifically, the very different rates of vertical Cs transport inferred from the distributions of ^{134}Cs and ^{137}Cs cannot be resolved. Downward smearing could not enhance the apparent mixing rate of one Cs isotope without similarly affecting the distribution of the other. This inconsistency remains an enigma.

The known and suspected processes of sediment focussing, sediment mixing, diffusion in pore waters, and core smearing did not cause the xs^{210}Pb distributions to deviate measurably throughout most of the sediment record from a log-linear pattern which is usually taken to indicate a well-preserved profile and an accurately recorded accumulation rate. As demonstrated here, perfect fits of xs^{210}Pb profiles to log-linear distributions do not guarantee the validity of the sedimentation rates calculated from the xs^{210}Pb profiles (see also Robbins et al. 1978).

Sediment accumulation rates calculated from xs^{210}Pb gradients agreed within a factor of two with accumulation rates for these lakes determined by independent methods. Accumulation rates calculated from xs^{210}Pb gradients also agreed fairly well with rates calculated by an independent method that uses ^{210}Pb data (see Equation 15 and Table 3). However, these independently derived rates are not well enough constrained to discount errors of up to a factor of two for the accumulation rates derived from the xs^{210}Pb gradients.

Accurate sediment chronologies are critical for dating events recorded in lake sediments. Excess ^{210}Pb chronologies are applicable over a time scale of about a century, which is ideal for dating many of the effects of human perturbation of the environment. For example, the history of acidification of lakes has been reconstructed from diatom records in lake sediments (e.g. Dickman et al. 1984). Problems of the type identified here could cause erroneous dating of the microfossil records. These problems may be less serious for other systems where sediments have lower porosities than the lakes in this study. However, until the reasons for the problems are identified, and because these problems were not evident in the commonly measured ^{210}Pb profiles, we must urge caution in accepting chronologies based entirely on ^{210}Pb results. Confirmation by two independent methods should be a minimum criterion for accepting the validity of sediment chronologies.

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Validating the Use of Radiotracers as Analogs of Stable Metal Behaviour in Enclosed Aquatic Ecosystem Experiments

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Radiotracer experiments were carried out in enclosures in two lakes at the Experimental Lakes Area, Northwestern Ontario, to study the pathways of trace metal removal from the shallow water columns to the sediments of these lakes. As part of the larger experiment, stable carriers of Fe, Mn, and Zn were added individually to each of three enclosures. Rates of removal to sediments at pH 6.3 and backdiffusion after HCl addition to pH 4.8 of radiotracer ^{59}Fe, ^{54}Mn, and ^{65}Zn were compared to the respective rates of their stable counterparts. Tracer removal and backdiffusion rates in enclosures which received stable carrier additions were further compared with the tracer behaviour in enclosures which received no carrier additions. Radiotracers and stable carriers were removed from the water column at similar rates at pH 6.3, and were remobilized at similar rates from the sediments when the pH of the water column was lowered to 4.8. The presence of the added stable carriers had no effect on the removal or remobilization of Mn or Zn. However, the removal of ^{59}Fe from the water column was slowed slightly by the addition of Fe(III) carrier compared to removal in the other tubes, possibly due to the formation of colloidal phases. These results show that information on the rates of trace metal transport obtained using radiotracers is applicable to stable metal behavior in these freshwater systems. The use of radiotracers to study trace metal geochemistry under natural conditions is preferred to the addition of stable metals which may alter the physical and chemical properties of the suspended particulate matter as occurred after the addition of stable iron. Speciation studies showed that adsorption to ion exchange resins does not provide a reliable indication of the ionic form of metals in lake water.

Des essais avec traceurs radioactifs ont été menés en enceintes dans deux lacs de la Région des lacs expérimentaux, dans le nord-ouest de l'Ontario, en vue de connaître les voies d'enlèvement des métaux en traces des colonnes d'eau de faible profondeur de et transfert dans les sédiments de ces lacs. Au cours d'une expérience plus considérable, des porteurs stables de Fe, Mn et Zn avaient été ajoutés individuellement à chacune des trois enceintes. Les taux d'enlèvement et de transfert dans les sédiments à un pH de 6,3 et de rétrodiffusion, après addition de HCl jusqu'à un pH de 4,8, des traceurs radioactifs ^{59}Fe, ^{54}Mn et ^{65}Zn ont été comparés aux taux respectifs de leurs contreparties stables. Les taux d'enlèvement et de rétrodiffusion des traceurs dans les enceintes où l'on avait introduit des porteurs stables ont été en outre comparés avec le comportement des traceurs dans les enceintes qui n'avaient pas reçu de porteurs. Au pH de 6,3, les traceurs radioactifs et les porteurs stables disparurent de la colonne d'eau à des taux identiques et furent enlevés des sédiments également aux mêmes taux quand le pH de la colonne d'eau était abaissé à 4,8. L'addition de porteurs stables n'eut pas d'effet sur l'enlèvement ou la remobilisation de Mn ou Zn. Cependant, l'enlèvement de ^{59}Fe de la colonne d'eau a été légèrement ralenti par addition d'un porteur de Fe(III) comparativement à ce qui s'est produit dans les autres tubes, probablement à cause de la formation de phases colloidales. Ces résultats démontrent que les données sur les taux de transport de métaux en traces obtenues à l'aide de traceurs radioactifs sont applicables au comportement des métaux stables dans ces systèmes d'eau douce. Dans des conditions naturelles, l'emploi de traceurs radioactifs dans des études géochimiques de métaux en traces est préférable à l'addition de métaux stables susceptibles se modifier les propriétés physiques et chimiques de la matière particulaire en suspension, comme cela s'est produit après addition de fer stable. Des études de spéciation démontrent que l'adsorption à des résines échangeuses d'ions n'est pas un indice fiable de la forme ionique des métaux dans l'eau des lacs.

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Many trace metals have been mobilized into the environment by our industrial society. Some of these metals (e.g. Pb, Hg, Cd) have no known biological requirements, whereas other trace metals (e.g. Cu, Ni, Zn, Co) are essential micronutrients, and metals in both catagories may become toxic even at low ppb concentrations (e.g. Phipps 1976; Hughes 1981; Anderson and Morel 1978; deNoyelles et al. 1980; Marshall and Mellinger 1980; Marshall et al. 1983; Peterson 1982). Therefore, it is important to understand what, and how, biogeochemical processes control the transport and fate of these metals in aquatic systems.

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It is difficult to study the biogeochemical cycling of trace metals in unperturbed ecosystems. Concentrations of the metals in solution are often very low. When metals are present at detectable but very low concentrations, contamination during sample handling becomes a serious problem (e.g. Bruland et al. 1979). Even when concentrations of the metals can be measured reliably, it is difficult to assess the rates and mechanisms of metal transport within the system because transport along many pathways is occurring simultaneously. Furthermore, simply measuring total particulate metal concentrations cannot distinguish between reactive and non-reactive (e.g., aluminosilicate or refractory oxide) particulate metal pools. Therefore, approaches other than the simple measurement of the distributions of trace metals in an aquatic system must be employed to identify and quantify the biogeochemical processes which determine the mobility and fate of metals in the system.

One approach to the study of trace metal geochemistry in aquatic ecosystems is to perturb the system by adding stable (nonradioactive) metals in a known chemical form. The response of the system to the perturbation can then be monitored to determine the fate of the added metal. Factors which would be investigated include the overall rate at which the concentration of metal in solution returns to its pre-spike level, the extent to which the metal partitions between solution and solid phases, incorporation of the metal into biota, and the sediment phases which finally incorporate the metal. However, some problems are encountered in following this approach. For example, there may be a substantial reservoir of some of the metals naturally present in the solid phases (e.g. Fe, Al). In order to measurably increase the concentration of the metal in the solid phases, the concentration in solution may first have to be raised by several orders of magnitude. Consequently, the behavior of the metal in solution under such elevated concentrations may not be representative of its behavior under natural conditions. For example, the behavior of the metal may be altered by biological stress on the system through the toxic effects of the metal on organisms. Solid phases not present naturally may be formed by precipitation of the added metal, and it is known that the affinity of a metal for adsorption to solid surfaces often decreases as the concentration of the metal in solutions increases (Benjamin and Leckie 1981; Balistrieri and Murray 1983, 1984). Therefore, it is essential that the chemical conditions of the system under study be perturbed as little as possible in investigating the behavior of trace metals.

A second approach to the study of trace metal geochemistry is to introduce radioactive tracers of the metals of interest into a natural aquatic ecosystem, or into an enclosed portion of a natural system. These tracers can then be followed as they proceed through various intermediate biological and geochemical phases to their ultimate geological repositories. This approach has been applied to the study of the fate of trace metals at the deep ocean sediment−water interface (Santschi et al. 1984), in enclosed coastal marine ecosystems (Adler et al. 1980; Santschi et al. 1980, 1982, 1983; Amdurer et al. 1982; 1983), in enclosed freshwater systems (Schindler et al. 1980; Jackson et al. 1980; Santschi et al. 1986), and in whole−lake freshwater systems (Hesslein et al. 1980; Hesslein 1987). In each of these studies, dissolved tracer was added in ionic form, and the incorporation of the tracers from solution into biota, into suspended particles, and into sediments was monitored.

Advantages of this approach include:

1) carrier-free tracers can be added without perturbing the natural levels of the metals of interest,
2) accurate budgets of the added tracer can be kept, and experimental controls can be maintained when the experiments are carried out in enclosed systems,
3) the distribution of the tracer can be followed throughout an entire ecosystem rather than studying one process at a time in "beaker experiments" where synergistic effects would not be evident,
4) labor requirements are minimized because several elements can be measured rapidly and simultaneously by gamma spectrometry with minimal sample processing, and
5) problems of contamination, which are serious when measuring natural low levels of trace metals, are avoided.

Although the use of radiotracers to study the geochemical behavior of trace metals is becoming widespread, there is still some concern that the tracers may not provide a valid representation of the rates and mechanisms of trace metal transport within natural aquatic systems. This concern arises because the tracer is added in one chemical form to one component of a complex natural system. Some of the apparent transport of the tracer subsequent to its addition to the system may then simply reflect short term isotopic exchange of the tracer with its stable metal counterparts in other components of the system. This isotopic exchange might lead to an incorrect evaluation of physical transport rates. In this paper we describe the results of an experiment to examine the validity of determining physical transport rates for trace metals using radiotracer results.

Materials and Methods

The results presented in this paper were obtained as part of a larger study of the transport and fate of trace elements in freshwater systems at the Experimental Lakes Area (ELA), Northwestern Ontario. Details of the experiment can be found in Santschi et al. (1986). Briefly, radioactive tracers of several elements were added to the water columns of 20 tubes (limnocorrals) in two ELA lakes. Individual tubes were manipulated in different fashions, while one or two control tubes were maintained in each lake. The tubes were constructed of nylon-reinforced polyethylene. The top of each tube was held in a 1 m diameter ring of plastic pipe attached to a wooden frame held 30 cm above the air−water interface by styrofoam floats.The water column depth of the tubes was about 1.2 m, and the bottom of each tube was embedded in the sediments.

Most of the results described in this paper are from tubes in Lake 114, a shallow (mean depth = 1.7 m) oligotrophic lake with highly porous, flocculent organic-rich (24% organic carbon; Kelly and Rudd 1984) sediments. Inorganic components of ELA lake sediments are primarily quartz, plagioclase, and K-feldspar in the sand and silt fractions, and illite and kaolinite in the clay size fraction (Brunskill et al. 1971). The pH of the epilimnion was $\simeq$6.3 and the total cation concentration was $\simeq 150\ \mu eq \cdot L^{-1}$ at the time of the experiments. Other characteristics measured one year after the experiment include:

alkalinity 27 $\mu eq \cdot L^{-1}$, sulfate 92 $\mu eq \cdot L^{-1}$, DOC 10 $mg \cdot L^{-1}$, and conductivity 22 μS.

Four tubes in Lake 114 each received additions of one stable metal in ionic form as chloride salts sufficient to raise the concentrations of these metals to 120 μg (2.15 μmol) $Fe^{3+} \cdot L^{-1}$ (Tube 14), 160 μg (2.91 μmol) $Mn^{2+} \cdot L^{-1}$ (Tube 15), 60 μg (0.92 μmol) $Zn^{2+} \cdot L^{-1}$ (Tube 16), or 13 mg (332 μmol) $K^{+} \cdot L^{-1}$ (Tube 17). The KCl additions to tube 17 raised the total concentration of cations in the water from about 150 $\mu eq \cdot L^{-1}$ to about 480 $\mu eq \cdot L^{-1}$ to test for any effect of increased competition for particulate sorption sites on the pattern of metal removal from the water column. Approximately $2-6 \times 10^6$ Bq of gamma emitting tracers were added to each tube in ionic form in small amounts (10's of μL) of HCl or HNO_3. The maximum perturbation of the natural trace metal concentrations resulting from tracer additions was for Zn, for which the maximum permissible amount of carrier in the tracer according to the manufacturer's specifications would have increased the Zn concentration in the tube water by about 5%.

The initial pH of the water column in Lake 114 was about 6.3. The pH of the control tube followed the general decrease of the epilimnion pH to about 6.1 in early August, after which the epilimnion pH remained nearly constant whereas the control tube pH continued to decrease until it stabilized at about 5.8. Eighteen days after spiking the tubes with the radiotracers, HCl was added to lower the pH of the tube water columns to about 4.8. The pH was monitored daily, and acid was added as necessary to maintain a pH of 4.8. Typically, 100–150 $\mu eq\ H^{+} \cdot L^{-1}$ were added during this stage (Santschi et al. 1986).

Unless otherwise specified, radiotracer results reported here are for 100 mL samples of unfiltered water integrated over the top 1 m of the water column. Other samples were collected routinely to monitor the total, the particulate, and the dissolved tracer activities (after filtration through prewashed and preweighed 0.4 μm pore diameter Nuclepore filters), and to provide the concentration of suspended particulate matter. Water samples and filters were gamma counted in standardized sample geometry on a Ge(Li) detector coupled to a 4096-channel pulse height analyzer. Appropriate geometry factors were determined by counting mixed isotopic standards in the same geometry configurations as were used for the samples.

Unfiltered water samples for stable trace metal analyses were collected in hand-held 125 mL polyethylene bottles prewashed with HCl and Milli Q water while wearing new disposable polyethylene gloves. Samples were preserved without further treatment by acidifying to $pH < 2$ with Aristar H_2SO_4, after which the sample bottles were enclosed in double plastic bags to prevent contamination during transport back to the lab. Analyses were carried out by direct injection graphite furnace atomic absorption spectrometry.

The natural concentrations of trace metals in ELA lakes are very low (e.g. $\simeq 1$ ppb Zn, <0.06 ppb Pb, <0.1 ppb Ni, <0.15 ppb Co, $\simeq 0.3$ ppb Cu and $\simeq 0.03$ ppb Cd; Anderson, unpublished results), making it extremely difficult to manipulate samples without imposing contamination artifacts. Several of the tubes in the second lake (Lake 302) had closed bottoms which were covered with a layer approximately 5 cm deep of sediments brought in from other lakes (Santschi et al. 1986). One of these lakes (Clearwater Lake near Sudbury Ontario) had sediments contaminated with high concentrations of metals transported through the atmosphere from a nearby smelter (Yan 1979). The water column of this tube had metal levels high enough (14 ppb Zn, 13 ppb Mn, and 2.5 ppb Co in water filtered through a 0.4 μm Nuclepore filter) that the samples could be manipulated to compare the speciation of added radiotracers with the speciation of their stable counterparts derived from the sediments.

Water samples from this tube which had been filtered through a 0.4 μm Nuclepore filter were run through parallel pairs of ion exchange columns in series. One series consisted of an anion exchange column (Dowex AG 1-X8) preceding a cation exchange column (Dowex AG 50W-X12) while the other series had the cation column preceding the anion column. Columns, resins, and other labware were washed with reagent grade HCl and then with distilled-deionized (Milli Q) water to remove trace metal contaminants. Metals retained by each column were eluted with concentrated HCl, so that for each series we obtained fractions which were identified as anion (retained by anion resin), cation (retained by cation resin), and neutral (not retained by either resin). An identical set of fractions was collected for blank analysis by filtering Milli Q water and running it through the series of columns.

Results

The precision of stable trace metal analyses varied with sample concentration and from day to day with instrument performance. Reproducibility of absolute concentrations on different days was 10–20%. However, all of the samples from each tube were analyzed for each metal together in a batch where the relative concentrations were known to ±2–5%. As the relative change in concentration with time is used to quantify the transport rates, this precision is also a measure of the accuracy of the rate constant determinations. Blank corrections for the results reported in this paper were insignificant except for stable Zn, for which the maximum blank correction in samples from the unspiked tubes was about 20%.

Equal amounts of each tracer were added to each tube. However, the volume of the enclosed water column varied slightly from tube to tube, making it difficult to compare absolute concentrations of tracer in the water column. To facilitate comparison of results between tubes, we present normalized results in which the measured concentrations of each tracer on each sampling day has been divided by its concentration on the first day after spiking. All results are then presented as the fraction of the initial concentration (percent) remaining in the water column on each sampling day.

Measurable amounts of each of the added stable metals were naturally present in the water of the unspiked tubes. It was therefore necessary to correct for the background levels of each metal before comparing radiotracer behavior with the behavior of the corresponding added, or excess, stable carrier. Fe, Mn, and Zn were each measured in at least two tubes. The concentration of added stable metal present on each sampling day was calculated by subtracting a running mean concentration of the metal in one or two unspiked tube(s) from the concentration in the spiked tube. Natural Fe, Mn, and Zn concentrations varied from day to day, so the variability about the running mean was included in propagating the uncertainties of the calculated excess metal concentrations. This additional uncertainty was greatest for Fe, for which the difference between natural and spiked concentrations was the smallest, and least for Zn. These corrected concentrations were then normalized by dividing by the initial concentration as described above for the radiotracers and plotted with the

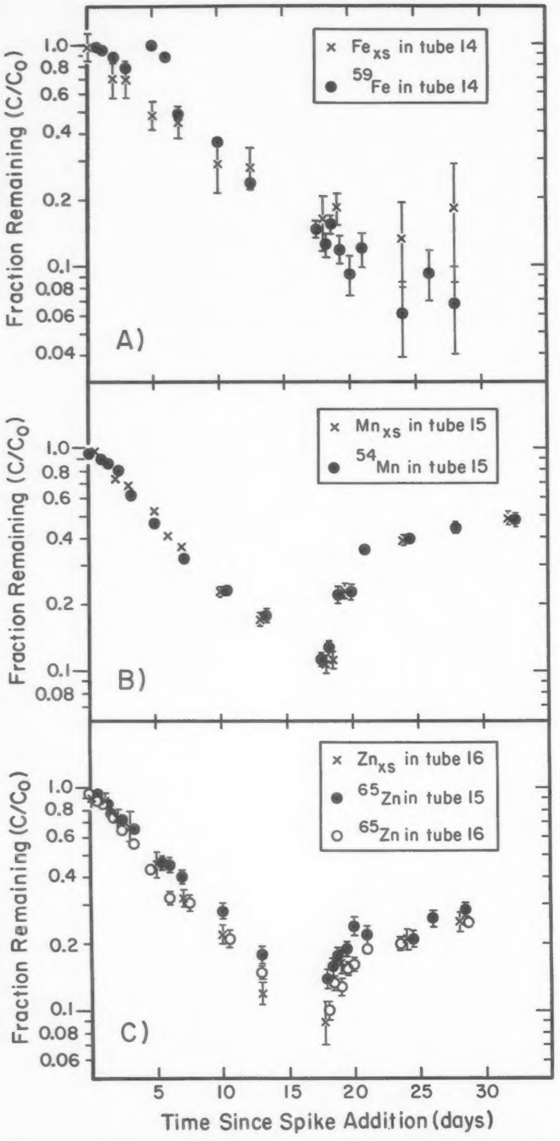

FIG. 1. Fraction (percent) of tracer ^{59}Fe, ^{54}Mn, and ^{65}Zn and of excess (total minus background concentration) stable Fe (A), Mn (B), and Zn (C), initially added to the water column of three tubes in ELA Lake 114, present in the water column as a function of time after spiking. Day zero is 3 July, 1981. Error bars for radioisotopes represent one standard deviation of counting statistics propagated through the normalization procedure described in the text. Error bars for stable isotopes represent the propagated uncertainties from the analyses of the metal concentrations in the spiked and in the unspiked tubes. The increased ^{59}Fe concentration on days 5 and 6 (A) resulted from resuspension of some surface sediments during a severe wind storm. The water columns of the tubes were acidified with HCl from pH 6.3 to pH 4.8 on day 18. All samples were unfiltered.

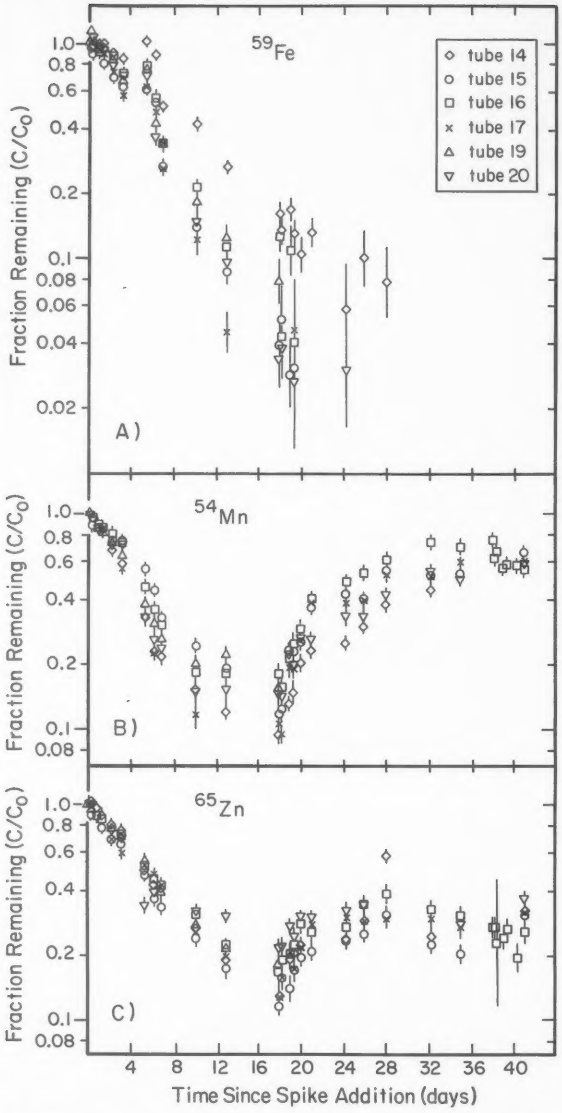

FIG. 2. Fraction (percent) of radioactive ^{59}Fe (A), ^{54}Mn (B), and ^{65}Zn (C) initially added to the water columns of six tubes in ELA Lake 114 present in the water columns as a function of time after spiking. Tubes 14–17 received additions of stable Fe, Mn, Zn, and K, respectively, as described in the text. Tubes 19 and 20 served as controls. Error bars represent one standard deviation of counting statistics propagated through the normalization procedures described iin the text. The water columns of the tubes were acidified with HCl from pH 6.3 to pH 4.8 on day 18. All samples were unfiltered.

radiotracers results to provide a direct comparison between the behaviors of the tracers and the carriers in the water column. Comparisons between stable metals and radiotracers in the particulate phase were not made because of the potentially large, and unquantified, concentrations of unreactive (e.g. bound within aluminosilicates or refractory oxides) metals naturally present in the particles and sediments.

For both the tracer and stable forms, 90 ± 3% of each of the Fe, Mn, and Zn were eventually removed to the sediments at pH 6.3 (Fig. 1). Of the metals removed to the sediments by

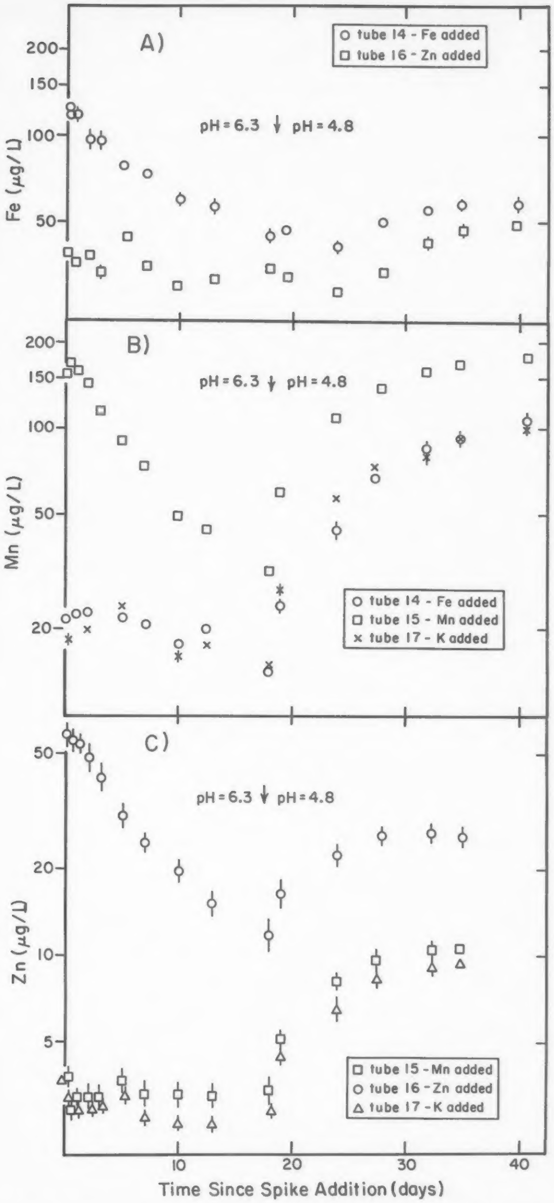

FIG. 3. Concentrations of stable Fe (A), Mn (B), and Zn (C) in Lake 114 tubes. Data are plotted on a semi-log scale for comparison with Fig. 1 and 2. The water columns of the tubes were acidified with HCl from pH 6.3 to pH 4.8 on day 18. All samples were unfiltered. Where error bars are not shown, they are the size of the symbols or smaller.

day 18, 50% of the Mn (both stable and tracer) and 30% of the Zn (both stable and tracer) were remobilized to the water column during the 2 wk following acidification to pH 4.8 (Fig. 1). For each of the metals, there was no significant difference ($P < 0.05$) between the removal rates of the radiotracers and of the stable carriers. A detailed discussion of the removal rates, and of the physical processes which determine them, is beyond the scope of this paper and is presented elsewhere (Santschi et al. 1986). Analytical uncertainty in the ^{59}Fe measurements was poor toward the end of the experiment because only a small fraction of the ^{59}Fe remained in the water column and because much of the ^{59}Fe had decayed before the samples could be counted.

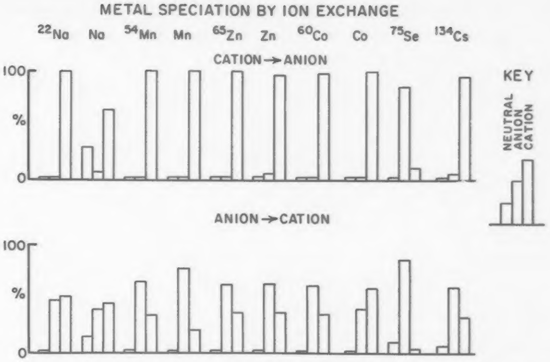

FIG. 4. Results of a speciation experiment in which filtered (0.4 µm) water from a tube containing metal-contaminated sediments was passed through two series of ion exchange columns. The top bar graph shows the results when water was passed through a cation column followed by an anion column. The order of the columns was reversed for the bottom graph. Results are presented as percent of the total metal concentration present in each of three fractions: that which was retained by the anion resin, that which was retained by the cation resin, and that which passed through both columns. Results are presented for both radioactive and stable Na, Mn, Zn, and Co, as well as for radioactive Cs and Se. All radiotracers except Se were added exclusively as cations.

Within the variability of the results there are no systematic, or significant ($P < 0.05$), differences in the removal rates of ^{54}Mn or ^{65}Zn between any of the six tubes (Fig. 2B,C). In contrast, the removal rate of ^{59}Fe (Fig. 2A) was significantly slower (half removal time 6.26 ± .20 d) in Tube 14 which received Fe(III) carrier than in any of the other five tubes. There was no significant difference between the ^{59}Fe removal rates in the other five tubes (average half removal time 3.56 d; range 2.92 to 3.93 d; maximum 1 σ of ± 0.37 d). The variability of the data points is partly due to sampling and analytical uncertainty and partly the result of small intertube variations in the rate of one or more of the transport processes which affect metal removal and remobilization (Santschi et al. 1986), including the concentration and settling rate of particles, the turbulence in the water column, and the rate of sediment mixing.

Natural stable Fe, Mn, and Zn concentrations in the unspiked tubes remained fairly constant over the first 18 d (pH = 6.3) of the experiment (Fig. 3). After acidification on day 18, the stable Fe, Mn, and Zn concentrations began to increase in the unspiked tubes, as well as in the spiked tubes, indicating that naturally occurring metals were being mobilized by the acid. This effect was less for Fe (Fig 3A) than for Mn or Zn (Fig 3B, 3C).

Results of the speciation experiment using water from the tube to which Clearwater Lake sediments were added showed generally similar distributions of the radiotracers and the stable metals within each experiment (Fig. 4). However, the apparent distribution between anionic and cationic forms for most elements differed greatly depending on the order of the ion exchange columns. The bar graphs represent for each species the per cent of the total recovered metal appearing in the uncharged, anion, and cation fractions as described in the methods section. Blank corrections amounted to <10% for Co, 10–20% for Mn, and 20–40% for Zn for fractions containing the eluted metals. For other fractions, the metal concentrations were at blank levels. The blank fractions were also gamma counted to prove that we had no unknown blanks, for example by cross contamination from other samples or from a contaminated detector. Samples from other tubes were processed similarly, but the stable metal concentrations were too low to be measured accurately.

Discussion

Removal of Metals from Water to the Sediments

Two principle criteria were established for accepting the validity of transport rates obtained from radiotracer results.

1) A radiotracer and a stable carrier of a specified element added to the water column simultaneously must be removed from the water column at similar rates.

2) Radiotracer removal rates must not be affected by the concentration of the stable metal in solution providing that the concentration of the stable metal is not elevated to the point that new solid phases are precipitated or that sorption sites of natural particulate matter become saturated (Benjamin and Leckie 1981).

If these criteria are observed to hold in our experiments then we can conclude that tracer removal rates are not biased by isotopic exchange reactions, and that the metal transport rate information derived from the tracer studies is intrinsic to the system under study. These transport rates could then be used to calculate the rate at which the system would return to natural condition if perturbed, for example by the input of pollutants. Santschi et al. (1986) have shown that removal rates depend, among other factors, on the bioturbation rates in the sediments, and on the kinetics and extent of sorption at the particular pH value. They showed that metal removal rates were consistent with these parameters, each of which was determined independently.

Removal rates of tracer and stable Fe, Mn, and Zn were indistinguishable within the analytical uncertainty of ±10–20%, depending on the element (Fig. 1). The best agreement was observed for Mn which also had the smallest relative uncertainty. Similar behaviors of tracers and stable carriers for each element shows that the first criterion listed above was satisfied.

The second criterion, that tracer removal is not affected by varying the concentration of its stable carrier, can be examined using results in Fig. 2. There were no significant or systematic differences in the removal rates of ^{54}Mn or of ^{65}Zn in any of the tubes. This means that the processes transporting these metals between the water column and the sediments are not sensitive to the concentration of the metal over the range between the ambient levels in the lake and the concentration of the added carriers. Furthermore, the change in ionic strength by a factor of three had no effect on the removal of ^{59}Fe, ^{54}Mn, or ^{65}Zn. Therefore, the second criterion for accepting the validity of tracer results holds for Mn and Zn, and the rate constants for removal of these tracers to the sediments determined during these experiments (Santschi et al. 1986) could be multiplied by the appropriate concentration of each of the stable metals in the lake water column to establish the flux of each metal from the water to the sediments under the conditions (pH, particle flux, sediment mixing rate, etc.) occurring at the time of the experiment.

The fraction of ^{59}Fe remaining in the water column of Tube 14 ($FeCl_3$ added) was systematically higher than the fraction of ^{59}Fe remaining in the other tubes throughout the experiment (Fig. 2A). Consequently, the half removal time for ^{59}Fe in Tube 14 (6.26 ± .20 d) was significantly greater than the average of the other five tubes (3.56 d) as well as the individual tube (15) having the next longest half removal time (3.93 ± 0.37 d). One possible explanation for the slower removal of ^{59}Fe in Tube 14 is that the added $FeCl_3$ hydrolyzed and formed colloidial $Fe(OH)_3$ particles which settled more slowly than the particles naturally present in the system because they were stabilized by association with dissolved organic matter (Sholkovitz 1976; Boyle et al. 1977; Loder and Liss 1985). These $Fe(OH)_3$ particles must then have carried a significant portion of the ^{59}Fe without affecting the other tracers since there was no systematic difference (within 2 sigma) between the removal rates of ^{54}Mn or ^{65}Zn in Tube 14 and their removal rates in the other tubes (Fig. 2B, C). This behavior of Fe in Tube 14 provides a good illustration of the type of problem which may be encountered when trying to study trace metal geochemistry by increasing the concentration of the stable metal in solution. Some process related to the addition of Fe carrier decreased the rate of Fe removal from the water column. Had these experiments been carried out using stable metals rather than radiotracers, we would have incorrectly evaluated the transport parameters removing Fe, and possibly other elements, from the water column (Santschi et al. 1986).

Mobilization of Metals by Acidification

It is evident in Fig. 1 and 2 that Mn and Zn added to the water columns of the tubes and removed to the sediments at a pH of about 6.3 were remobilized back into the water column when the pH was reduced to 4.8. The reasons for this remobilization are discussed in detail elsewhere (Santschi et al. 1986). Stable Mn and Zn responded quickly to acidification in both the spiked and the unspiked tubes (Fig. 3B, C), with an increase in the water column concentration being clearly evident one day after acidification, and then levelling off toward the end of the data record. The rapid response of Mn and Zn to acidification suggests that the concentrations of these elements in the water column are controlled by a pH dependent chemical exchange equilibrium with the surface sediments. It is therefore unlikely that Mn^{2+} oxidized to Mn(IV) to a significant degree during the experiment since dissolution of MnO_2 would not have responded so quickly to a decrease in pH. The tendency for Mn and Zn concentrations to approach a new steady state value within 2–3 wk after acidification also suggests that the concentrations of these elements are controlled by exchange processes with surface sediments, and that the kinetic time constants of the exchange reactions are on the order of several days or less.

There are three important features of the Fe results (Fig. 3A): (1) There was an acid-induced mobilization of the added stable Fe which could not be detected using ^{59}Fe because its short half life prevented reliable results from being obtained late enough into the experiment; (2) The concentration of Fe in

Tube 16 increased slightly, indicating mobilization of Fe naturally present in the sediments; (3) Mobilization of stable Fe in both Tubes 14 and 16 did not occur until about 10 d after acidification, unlike the immediate response observed for Mn and Zn. A complete explanation for this delayed response of Fe requires further study, but it could indicate that the dissolution of solid phase Fe is a much slower process than the desorption or exchange of Mn^{2+} and Zn^{2+} resulting from H^+ addition.

It is possible to quantify the amounts of the added stable metals that were remobilized by lowering the pH from 6.3 to 4.8, and to compare these results with the extent of remobilization of the corresponding radioactive tracers. We calculate from the results in Fig. 3 that 50, 30, and 10% of the added stable Mn, Zn, and Fe, respectively, which had been removed to the sediments at pH 6.3 were later remobilized to the water column after lowering its pH to 4.8. Similarly, 50% and 30% of the ^{54}Mn and ^{65}Zn, respectively, which had been removed to the sediments at pH 6.3 were remobilized to the water column at pH 4.8 (Fig. 1B, C). The similar behavior of radiotracers and stable carriers shows that in an aquatic ecosystem with close coupling between the water column and the sediments, radioactive and stable metal ions equilibrate quickly and behave very similarly.

The question of the validity of tracer results arises again in considering the remobilization of trace metals by acidification. Both the radiotracers and the stable metals added to our tubes had been present in the system for only 18 days when the water columns were acidified. If the tracers remained in a more labile chemical form than the metals naturally present, then our results would overemphasize the effect of acidification on the mobilization of trace metals from the sediments. Unfortunately, we cannot determine the rate constants for mobilization of the trace metals naturally present because we do not know the size of the sediment reservoir of potentially available metals. We can, however, demonstrate a significant increase as the result of acidification in the water column concentrations of natural Mn (Fig. 3B, Tubes 14 and 17), Zn (Fig. 3C, Tubes 15 and 17), and probably Fe (Fig. 3A, Tube 16). We can further demonstrate that the responses of the naturally occurring metals were qualitatively similar to the responses of the radiotracers and the added stable metals. Mobilization of Fe in Tube 16 from $32 \pm 5\ \mu g \cdot L^{-1}$ to $50 \pm 5\ \mu g \cdot L^{-1}$ (Fig. 3A) was both slow and relatively small compared to the day to day variability in the concentration of Fe, similar to the slow remobilization of about 10% of the added Fe carrier only after 10 d in Tube 14. In contrast to Fe, mobilization of naturally present Mn in Tubes 14 and 17 was evident within 1 d of water column acidification, and proceded to a level of about five times the initial Mn concentration (Fig. 3B). Mobilization of naturally occurring Zn was also evident within 1 d of water column acidification, with the Zn concentration levelling off at about three times its initial value (Fig. 3C). The rapid response of Mn and Zn compared to the slow responce of Fe, the rates at which new steady state values of metal concentrations were approached, and the relative nagnitude of mobilization (Mn>Zn>>Fe) for naturally occurring metals, were all very similar to the responses of the added radiotracers and stable carriers. Therefore, even though rate constants for mobilization of naturally occurring metals cannot be determined, the similarities indicate that radiotracer results can be extrapolated to the behavior of naturally occurring metals in the sediments as well as in the water column.

Speciation

A question that arises when using radiotracers to study trace metal behavior in aquatic systems is whether or not the tracer reaches chemical equilibrium with the natural stable metal in solution before a significant amount of the tracer has been removed to particles or sediments. Ideally this would be tested by determining the speciation of both the tracer and the stable metal to demonstrate that the two are identical.

One approach which empirically constrains, without completely identifying, the speciation of metals in solution, is to pass filtered water samples through a series of ion exchange resins (Alberts et al. 1977; Alberts and Wahlgren 1981; Cleveland and Rees 1981; Wahlgren and Orlandini 1982). Using this technique, previous investigators have separated the total dissolved concentration of an element into anionic, cationic, and uncharged fractions. We followed this approach to compare the chemical forms of added radiotracers with those of stable metals naturally present. As described in the Materials and Methods section, only one tube containing sediments from Clearwater Lake had metal concentrations high enough to do this.

If the ion exchange separation technique gives a reliable measure of the charge of metals in solution, then the same results should be obtained whether the sample is first passed through an anion resin followed by a cation resin, or if the order of the resin is reversed. Results in Fig. 4 show that very different apparent speciations were obtained depending on the order of the columns. When the sample was passed through the cation column first, only for ^{75}Se (which should be present as an oxyanion) did we find most of the tracer appearing in the anion fraction. Nearly 100% of each of the other tracers was present in the cation fraction, as would be expected if these elements were present in their common ionic form. The apparent fraction of stable Na in the effluent arose from the blank contribution of the cation resin itself, which was used in the sodium form.

When the sample was passed through the anion resin first, substantial portions of every element appeared to be anionic, including $^{22}Na^+$ and $^{134}Cs^+$ which were added as cations. Similar results were described by Amdurer et al. (1982, 1983) who used sequential charcoal and chelex columns to extract radioactive trace metals from seawater. This was explained by the occurrence of the tracers in a colloidal fraction which was taken up by either column. These results demonstrate that passing filtered water samples through a series of ion exchange resins is not a reliable method for determining the speciation of trace elements. However, despite this problem, we did find similar distributions of Na, Mn, Zn, and Co in the three fractions for the tracers and for the stable metals, with the exception of the Na blank noted above. This suggests that the tracers and the stable metals had similar speciation, although the identity of the chemical fractions have not been determined unequivocally.

In the references cited previously (Alberts et al. 1977; Alberts and Wahlgren 1981; Cleveland and Rees 1981; Wahlgren and Orlandini 1982), samples were passed through an anion resin followed by a cation resin, and in each of these references the investigators came to the surprising conclusion that plutonium was largely in anionic form. Our results suggest that the plutonium speciation determined by these investigators should be reexamined.

Occurrence of trace metals (including Pu) in colloidal forms which are retained by many types of surfaces (anion, cation, and chelex resins as well as charcoal) would explain our results as well as those of the Pu studies mentioned above and of Amdurer et al. (1982, 1983). Empirical speciation studies conducted during earlier radiotracer experiments as ELA (Schindler et al. 1980; Hesslein et al. 1980) where filtered water samples were passed thtough charcoal columns followed by ion exchange columns may have similarly misidentified colloidal forms of the metals as dissolved organic complexes. Further studies of the physical form and chemical reactivity of trace metals in lakes are needed to resolve these problems.

Conclusions

Rate constants for transport of trace metals in aquatic systems obtained using radiotracers are applicable to stable metals. Furthermore, this rate information is applicable over a range of metal concentrations with the limitation that the physical and chemical properties of the solid phases present in the system are not altered. The fact that the amounts of the added radioactive and stable metals which were removed to the sediments and later remobilized by the acid, and the rates of these processes, were very similar demonstrates clearly that radioactive nuclides actually do replicate removal and remobilization processes as they affect stable metals, rather than simply reflecting "isotopic equilibration" effects.

An ion exchange technique for determining the speciation of trace metals in natural waters has been shown not to provide reliable fractionation of the different chemical forms, although the results were very similar for radiotracers and stable metals.

Mobilization of Fe, Mn, and Zn naturally present in the sediments of Lake 114 occurred as the result of the acidification of the water column from pH 6.3 to pH 4.8. The effective solubilities of many trace metals are controlled by adsorption to mineral surfaces and by coprecipitation with Fe and Mn oxyhydroxides (Jenne 1968; Turekian 1977; Huang et al. 1977; Hem 1979). Adsorption to natural mineral surfaces is pH dependent over the range of 4.8 to 6.3 for many metals (see review by Kinniburgh and Jackson 1981) and we have just shown that Fe and Mn can both be mobilized from sediments by acidification. Therefore, we can expect the concentrations of other trace metals to increase as the result of lake acidification as we have demonstrated for Fe, Mn, and Zn.

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Methods and Feasibility of Using Aluminum-26 as a Biological Tracer in low pH Waters

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Playle, R. C. 1987. Methods and feasibility of using aluminum-26 as a biological tracer in low pH waters. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 260–263.

The fate of aluminum accumulated by fish can be studied using ^{26}Al as a tracer, but the present cost of ^{26}Al will keep experiments to a small scale and replication to a minimum. The methods developed for liquid scintillation counting of ^{26}Al uptake will be most useful with exposures to low Al concentrations, where changes in Al concentration cannot be measured using other methods. Preliminary results showed that the gills and whole bodies of redbelly dace (*Chrosomus eos* Cope) accumulated more Al, when exposed to 480 $\mu g \cdot L^{-1}$ Al at pH 5.1, than dace exposed to 200 $\mu g \cdot L^{-1}$ Al at the same pH.

On peut étudier le sort de l'aluminum accumulé dans les poissons à l'aide de ^{26}Al comme traceur, mais, compte tenu du prix actuel de cet isotope, les essais seront à petite échelle et la réplication minimale. Les méthodes mises au point pour les mesures par scintillation liquide de captation de ^{26}Al seront très utiles avec exposition à de faibles concentrations d'Al dans les cas où les changements de concentration de cet élément ne peuvent être mesurés par d'autres méthodes. Les résultats préliminaires indiquent que les branchies et le corps entier du ventre rouge du nord (*Chrosomus eos* Cope) accumulent plus d'Al quand le poisson est exposé à 480 $\mu g \cdot L^{-1}$ Al au pH de 5,1 que quand il est exposé à 200 $\mu g \cdot L^{-1}$ Al au même pH.

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Aluminum liberated from terrestrial soils (Cronan and Schofield 1979) and lake sediments (Schindler et al. 1980) is potentially toxic to fish in lakes acidified by acidic precipitation (Cronan and Schofield 1979; Baker and Schofield 1980, 1982; Muniz and Leivestad 1980a). This toxicity occurs at pH values of 4.5 to 5.5 which otherwise may not be acutely toxic to fish. The site of toxic action of Al is assumed to be the gill, but it is unclear whether Al precipitates on the gills, impairing gas-exchange, or indirectly affects salt balance within the fish (Muniz and Leivestad 1980b; Brown 1983).

The purpose of this study was to develop radiotracer methods using ^{26}Al for studying Al accumulation in fish exposed to low concentrations of Al, when Atomic Absorption Spectrophotometry or Instrumental Neutron Activation Analysis may not be useful methods. The ^{26}Al isotope has not previously been used in biological studies. The influence of Al concentration on Al accumulation was briefly examined in an experiment run at 200 and 480 $\mu g \cdot L^{-1}$ total Al, near pH 5, which is not lethal to redbelly dace in 10 h. These concentrations of Al reflect realistic Al levels in lakes with pH of about 5 (see Muniz and Leivestad 1980a).

Materials and Methods

The long-lived isotope of ^{26}Al was discovered by Simanton et al. (1954) but its cost has apparently prevented its application to biological work. Commercial ^{26}Al costs about \$0.50 (U.S.) per Bq (\$18 000 per μCi). A small amount of ^{26}Al (1660 Bq; ratio of ^{26}Al atoms/^{27}Al atoms = 1/16) was purchased from Oak Ridge National Laboratory, Oak Ridge, TN. An absolute ^{26}Al standard (174 Bq; Standard Reference Material 4229) was obtained from National Bureau of Standards. Both ^{26}Al samples were measured on a Ge-Li detector (Ortec) and were free of other gamma-emitting isotopes.

For the ^{26}Al exposure experiment done in the summer of 1984, redbelly dace (*Chrosomus eos* Cope) were caught in minnow traps placed in Lake 114, the Experimental Lakes Area (ELA), northwestern Ontario, Canada, and were held overnight in flowing water from Lake 239. Individual dace were then placed in 90 mL of Lake 239 lake water in glass beakers. Each beaker contained about 114 Bq ^{26}Al (6830 disintegrations per minute; dpm) and stable Al added as $AlCl_3 \cdot 6H_2O$. The beakers were not aerated, and were placed in a water bath of flowing Lake 239 water which kept the temperature to ± 1°C of the lake's epilimnetic temperature. Cloth screening held in place by elastic bands prevented the dace from escaping. Fish were about 5 cm long and weighed approximately 1.2 g (wet weight). Placing 1.2 g fish in such a small volume of water (90 mL) was necessary because of the small amount of ^{26}Al available for the experiments.

The pH in each beaker was adjusted to 5.1 every 45 to 60 min with HCl or NaOH diluted in Lake 239 water. Exposure time was 10 h to allow time for Al accumulation to reach a steady state: previous research showed that Al removed from solution by minnows (*Notropis cornutus* (Mitchell)) was taken from solution over a period of 6 or 7 h (J. R. Kramer and R. C. Playle, Department of Geology, McMaster University, Hamilton, Ont., unpubl. data). All fish appeared healthy and none died during any experiment, although some surface gulp-

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TABLE 1. Summary of a ^{26}Al experiment using redbelly dace. The experiment was run for 10 h with 2 fish per test condition in separate 90 mL beakers. The amount of ^{26}Al added to each beaker was 6830 dpm. Aluminum accumulations have been rounded to one decimal place.

Experimental medium	Al exposure ($\mu g \cdot L^{-1}$)		Gill			Body		
	Initial	Final	dpm[a]	Dry weight[b] (g)	Al accumulation ($\mu g \cdot g^{-1}$)[c]	dpm[a]	Dry weight[b] (g)	Al accumulation ($\mu g \cdot g^{-1}$)[c]
1. Lake 239 water, pH 5.1 ± 0.2 19 ± 1°C	(a) 211	200	2.0	0.005	1.1	32.9	0.34	0.3
			−0.7	0.005	nil[d]	15.1	0.24	0.2
	(b) 481	315	70.5	0.01	44.7	37.1	0.30	0.8
			367.9	0.005	466.4[e]	56.2	0.30	1.2[f]

[a] Background subtracted using 5 gill, 5 body preparations. Negative values resulted when a gill or body cpm value was lower than the background of about 79 cpm (gill) or 100 cpm (body).

[b] 0.005 g used if 0.00 g was measured on the balance.

[c] Determined by calculating the weight of Al represented by the gill or body dpm, and dividing by the gill or body dry weight.

[d] Cannot be calculated because of negative dpm value. Assumed to be zero for statistical purposes.

[e] $P < 0.10$, t-test using log $(x + 1)$ transformed data. The t-tests compared the pair of gill or body Al accumulations from treatment (b) with the pair from treatment (a).

[f] $P < 0.05$, t-test using log $(x + 1)$ transformed data, as above.

ing, likely due to low oxygen levels, was observed.

Average concentrations of major cations and anions in Lake 239 epilimnion water during the experiment were, in $mg \cdot L^{-1}$, Na^+, 1.5; K^+, 0.7; Ca^{2+}, 3.3; Mg^{2+}, 0.9; Cl^-, 0.5; and SO_4^{2-}, 5.9. Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were 5.3 and 1.8 $mg \cdot L^{-1}$, respectively. Alkalinity was 145 $\mu Eq \cdot L^{-1}$, pH was 7.1, and the concentration of Al in the epilimnion was 27 $\mu g \cdot L^{-1}$. Aluminum concentrations in test solutions were measured using the lumogallion method (Playle et al. 1982) with minor modifications.

After Al exposure, the fish were dissected as follows. Each fish was put live in fresh deionised water for one minute to remove loosely bound Al, placed on a cutting surface, and killed by striking the back of the head. The head was cut off and the body placed in a pre-weighed and labelled glass scintillation vial. The gill arches were removed and placed into another pre-weighed vial. The remainder of the head was placed into the first vial with the rest of the body. Scalpel, tweezers, and the cutting surface were wiped clean before the next minnow was dissected. Each sample vial was weighed and then dried overnight at 85°C. Mean wet weight of the gills was 0.04 g. Weights were measured to 0.01 g accuracy on a Mettler P1200 top-loading balance.

Each vial was re-weighed after drying, placed in a muffle furnace for 14 h at 500°C to ash the fish material, and weighed again. (Aluminum-26 was not lost during ashing: samples with 19 Bq ^{26}Al added to dace material before ($n = 2$) or after ashing ($n = 2$) differed by <2%.) Concentrated HCl (2 mL) was added to the grey ash, followed by 5 mL of deionized water. Usually only a few specks of ash remained undissolved after swirling. Next, 15 mL PCS fluor (Amersham Corporation) was added and the vial shaken vigorously. A translucent, countable gel was formed.

To avoid photoluminescence, all samples were kept in the dark for at least one hour before counting. Beta emissions were counted on a Beckman LS 2800 liquid scintillation counter. The samples were counted for 100 min or until the 2% two-sigma counting error was reached. The largest error on any gross count rate was 3%. The same set of five ashed gill and five ashed body preparations from unexposed fish was used for background correction throughout the experiments. Background counts of ashed dace gills (≤ 0.01 g ash) were about 79 counts per minute (cpm). Counts were approximately 100 cpm for ashed dace bodies (about 0.03 g ash).

Liquid scintillation with fluor added to the sample vials was used in this study on the basis of its high detection efficiency. Aluminum-26 is both a γ and β^+ emitter with main γ peaks at 1.13 and 1.81 MeV, and β^+ emission at 1.16 MeV (Lederer and Shirley 1978). All ^{26}Al disintegrations produce γ emissions but only 82% produce β^+ emissions. Aluminum-26 standards prepared in 5 mL deionised water, 2 mL HCl, and 15 mL PCS fluor yielded 82 to 84% of the expected disintegrations on the Beckman LS 2800 scintillation counter. Corrections for radioactive decay were not necessary because ^{26}Al has a very long half-life, 7.2×10^5 years (Lederer and Shirley 1978).

Gamma detection of ^{26}Al using a Beckman 8000 NaI well detector was tried after this preliminary study was completed. Counting efficiency was about 70% with this machine. A detector of this type would probably be useful for future ^{26}Al work because of its high counting efficiency and the ease of sample preparation — no sample digestion or fluor is required.

Results and Discussion

An experiment using different concentrations of Al indicated that about 114 Bq ^{26}Al per 90 mL of solution were enough to quantify the accumulation of Al on dace gills and bodies (Table 1). Only two dace were exposed to each test condition, and statistical analysis of results from each experiment was limited to comparisons of gill or body Al accumulations, expressed as $\mu g \ Al \cdot g^{-1}$ (dry weight). These values were determined by calculating the weight of Al that was represented by a sample's dpm, and dividing by the dry weight of the sample.

Accumulation of Al at the gill increased 40 to 400× at the 480 $\mu g \cdot L^{-1}$ Al exposure, compared to accumulation at the 200 $\mu g \cdot L^{-1}$ Al exposure (Table 1, $P < 0.10$, t-test). Body accumulation increased at the higher exposure by only 3 to 4× ($P < 0.05$, t-test). The large increases of Al accumulation at the higher Al exposure could have been due to precipitation: 480 $\mu g \cdot L^{-1}$ Al at pH 5.1 is in the range where Baker and Schofield (1982) found Al to be most toxic to white suckers and brook trout. They concluded that precipitation and coagulation of Al hydroxides on the gill was the toxic mechanism of Al in oversaturated solutions (300 to 500 $\mu g \cdot L^{-1}$ Al, pH 5.2 to 5.4). However, increased Al binding at the gills could also have been a result of increased mucus production in response to Al stress, and differences in mucus production could explain some of the variability in the amount of ^{26}Al picked up by individual dace exposed to the same test conditions. For example, one of the dace exposed to 480 $\mu g \cdot L^{-1}$ Al accumulated ten times more ^{26}Al on the gills than the other dace exposed to the same test conditions (Table 1, treatment b).

A general idea of where the Al was bound on the fish was obtained by dividing the fish into gill and body portions, but the process of accumulation, adsorption or absorption, cannot be determined here because the methods used cannot distinguish the two processes. Retention of radioactive Cd by perfused fish gills is, according to Pärt et al. (1985), probably due to absorption, not adsorption. In my experiments, it is likely that both absorption and adsorption of Al took place at the gills, while only adsorption may have occurred at the fish body, because usually there was more ^{26}Al bound to the gills, on a $\mu g \cdot g^{-1}$ basis, than to dace bodies. Detailed comparisons of the Al-binding abilities of gills and fish bodies should consider binding by fish mucus and the influence of membrane surface area.

Aluminum-26 as a tracer is a sensitive but expensive tool, and its main use will be with exposures to low Al concentrations where changes in Al concentration cannot be measured using other methods. The amount of Al accumulated by the gill or body ranged between 0.2 and 466.4 μg Al $\cdot g^{-1}$ dry weight (Table 1). The lowest value (0.2 μg Al $\cdot g^{-1}$) is about 500× the resolution obtained by Instrumental Neutron Activation Analysis of ^{28}Al, half-life 2.3 min, used by Havas and Hutchinson (1983) to measure Al loss during acid exposure to *Daphnia*. It is about 5× the resolution of Atomic Absorption Spectrophotometry with a graphite furnace, used by Buergel and Soltero (1983) for analysis of fish tissues from an Al-treated lake.

Future experiments could use ^{26}Al to examine the effects of varying Al concentration, pH, Ca, and citrate levels on ^{26}Al accumulation by fish. The latter three factors have been briefly investigated (Playle 1985) but the results were inconclusive, mainly because of the small amount of ^{26}Al available. The effects of these factors on actual transfer of ^{26}Al through the gills could be studied by using perfused gills (Pärt et al. 1985) or cannulated fish (Neville 1979). The time required to reach equilibrium between Al adsorption, absorption, Al exchange, and Al excretion could be determined by running ^{26}Al uptake experiments for different lengths of time. Unfortunately the cost of the isotope will limit both the scale of the experiments and treatment replication, even if the ^{26}Al is recycled (Playle 1985). I recommend using approximately double the activity of ^{26}Al used here (i.e. 230 Bq per 90 mL solution), which would give higher counts and less ambiguous results. Aluminum concentrations as low as 60 $\mu g \cdot L^{-1}$ could be studied with this amount. Gamma detection using a NaI well detector would probably be a good substitute for β^+ detection using a liquid scintillation counter, especially for small volumes of fish blood.

The methods developed here for fish should be applicable to other biological materials. Carefully designed experiments would be able to answer key questions about Al uptake and distribution in plants or animals. Kushelevsky et al. (1976) were able to show selective uptake of ^{28}Al by cell nuclei and DNA in the rat liver, but were limited by the short half-life of their isotope. The long-lived ^{26}Al isotope should make experiments such as theirs more practical.

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Method for Estimating Dry Weight of Freshwater Planktonic Crustaceans from Measures of Length and Shape

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We have developed a method of estimating the dry weight of planktonic crustaceans by using geometric figures of their approximate shape. Measurements of three dimensions are used in conjunction with the formulae to estimate volume, and then, on the basis of a number of assumptions, individual weights are estimated from these volumes. We verified the accuracy of the estimates by comparing them with measured weights of adult cladocerans and copepods and subsequently adjusting the formulae. These data are used to form weight–length relationships for cladoceran species and for developmental groups of copepod species. These weight–length relationships may be useful for estimating the biomass of zooplankters from other lakes when size and shape and method of preservation are similar to those of our organisms. For specimens of different size and shape and for different species, the geometric formulae may be useful.

Les auteurs ont mis au point une méthode pour évaluer le poids sec de crustacés planctoniques à partir de figures géométriques de leur forme approximative. Trois dimensions sont couplées avec les formules d'estimation du volume puis d'après certaines hypothèses, les poids individuels sont estimés à partir de ces volumes. Les auteurs ont vérifié la précision des estimations en les comparant avec des poids établis de cladocères et de copépodes adultes et en ajustant les formules en conséquence. Les données ainsi obtenues ont servi à l'élaboration de relations poids–longueur pour des espèces de cladocères et des stades de développement d'espèces de copépodes. Ces relations poids–longueur peuvent servir à estimer la biomasse zooplanctonique d'autres milieux lacustres quand la taille et la forme du zooplancton et la méthode de conservation sont semblables à celles de la présente étude. Dans le cas de spécimens de forme et de taille différentes et d'autres espèces, les formules géométriques peuvent aussi être utiles.

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Our long-term studies of chemical and biological stresses on about 15 whole-lake ecosystems or enclosed aquatic communities in lakes in western Manitoba and in northwestern Ontario at the Experimental Lakes Area (ELA) include analyses of impacts on zooplankton species composition and biomass (Lawrence 1980; Lawrence and Holoka 1983; Malley and Chang 1981; Malley et al. 1982; Schindler et al. 1985; Malley and Chang 1986; Lawrence and Holoka 1987) and on the size-structure of zooplankton communities.

Dry weights of specific life stages of planktonic species are generally unavailable. Workers often use dry weights averaged over large size ranges (Wilson and Roff 1973; Roff and Kwiatkowski 1977; Hawkins and Evans 1979; Makarewicz and Likens 1979; Yan and Strus 1980). These values are of limited use to other workers unless the size distribution and body shape of organisms being studied is the same. Regressions of dry weight on length are of more general use, but variation arising from differences in shape remains unaccounted for. The statistic r^2, which measures closeness of fit, ranges from 0.35 to 0.98 for copepods and from 0.64 to 0.98 for cladocerans in such weight–length relationship studies (reviewed by McCauley 1984). A number of factors, often not reported, are known to affect weight–length regressions. These include range of length or of other body dimensions, relative contributions of nauplii and copepodids to the length–weight relationship for copepods, sex ratios of adult and preadult forms, contribution of eggs or ephippia to female biomass (Dumont et al. 1975; Bottrell et al. 1976), variations in dry weight with season (Hawkins and Evans 1979; Culver et al. 1985; Geller and Müller 1985), and variation in body length with experimental treatment (Makarewicz and Likens 1979). Clearly, for greatest accuracy, estimates of dry weight of zooplankton individuals should be generated for each study.

We needed a simpler, faster, and more universally applicable method than the frequently used drying and weighing of unpreserved individuals or groups of individuals. The method had to be useful for preserved material and for very small organisms, e.g. nauplii and rotifers. Leaching during preservation may affect the weight of zooplankters, making direct weighing of formalin-preserved organisms less accurate (McCauley 1984).

In addition to direct weighing of dried zooplankters, their weight may be estimated indirectly from individual volume. There are two methods of estimating volume: firstly, the volumes of enlarged scale models can be measured by displacement in water (Sebestyne 1955, 1958; Lewis 1979); secondly, simple geometric figures can be assigned to various species (Ruttner-Kolisko 1977). This latter method has been used for rotifers.

We extend to planktonic crustaceans this use of geometric figures to estimate dry weight. Volumes of these figures are calculated from three-dimensional measurements. Making several assumptions, we can convert these volumes to dry weight.

Our method depends upon verification of the assumptions by drying and weighing larger zooplankters and comparing the actual dry weights to those estimated. In some cases we have subsequently adjusted the original geometric formulae to make them more accurate.

We report the geometric formulae for estimating weight from measures of three dimensions of five species of cladocerans and five species of copepods. These formulae may be useful to other workers under conditions we describe. We also provide methods of estimating dry weight in two ways, (1) length–dry weight relationships for cladocerans and copepods, and (2) average stage-specific dry weight values for 12 developmental stages of copepods and the average size measurements of the ogranisms in each stage. The growth patterns of copepods obtained by this method are compared with those from studies in which the zooplankton dry weight or caloric content was measured directly.

Methods and Materials

Sites and Methods of Sampling

Lakes of the Experimental Lakes Area, located in northwestern Ontario (Cleugh and Hauser 1971), are small (between 5 and 56 ha), low in specific conductivity (15–25 μmho·cm^{-2} at 25°C; Prokopowich 1979), and naturally oligotrophic (200–300 mg C·m^{-2}·d^{-1} annual average primary production). A number of lakes have been experimentally modified (Cruikshank 1984).

Live zooplankton were obtained from seven lakes in ELA using 53 μm mesh nets and preserved specimens were provided by the routine sampling program in Lakes 227 and 223. The zooplankton sampling methods for Lakes 227 and 223 are described by Chang et al. (1980 and 1981, respectively). Size measurements were made on preserved zooplankton of Lake 223 sampled during the ice-free season of 1974, prior to the initiation of acidification, or on those sampled in 1978 and 1980 during acidification. Zooplankton of Lake 227 were from 1974 to 1978 samples.

Selection of Geometric Figures and Calculation of Dry Weight

Initially, a simple geometric figure approximating the shape of the irregularly shaped zooplankton individuals as seen under the microscope was chosen for each species or life stage. Most often a form of the simple ellipsoid, $4/3\pi r_1 r_2 r_3$, was used where $r_1 = a = 0.5$ length, $r_2 = b = 0.5$ width, and $r_3 = c = 0.5$ depth.

Copepod nauplii were judged to be ellipsoidal but with appendages lying outside the figure. To account for the apparent volume of the appendages, the depth dimension (c) was modified: nauplii 1 to 4, $4/3\pi ab1.5c$; nauplius 5, $4/3\pi ab1.4c$; and nauplius 6, $4/3\pi ab1.25c$. All copepodid copepods were initially described by $4/3\pi ab1.25c$. All cladocerans were initially described by $4/3\pi abc$.

The volumes of these figures were calculated from measured dimensions. Measurements were made along the three principal axes, i.e., anterior–posterior (length), lateral (width), and dorso-ventral (depth) (Fig. 1) using a binocular microscope at magnifications of 31 to 200×. Measurements were made to the nearest 0.005 mm.

Lengths of copepods were measured from the furthest projection of the head to the insertion of the caudal setae; widths and depths were measured at the widest point on the body. Life stages of copepods were measured separately, as were sexes of adults. Although individuals in a sample were measured consecutively as encountered to obtain a random sampling of the population, those animals obviously contorted or flexed were ignored.

Lengths of cladocerans were measured from the furthest projection of the head process to the point of insertion of the caudal spine, except for *Holopedium gibberum* whose length was taken as the distance between the *setae natores* and the terminal claw of the post-abdomen; widths and depths were measured at the widest points on the body. The gelatinous sheaths of *H. gibberum* were not included in measurements.

Only organisms without eggs, embryos, and ephippia were measured. Dry weight (biomass) of eggs were determined separately but are not reported here. The calculated volumes were converted to wet weight by assuming that 1 mm^3 weighs 1 mg, and thence to dry weight assuming the dry weight for all species to be 7% of live weight, a value adopted after considering Nauwerck (1963), Schindler and Novén (1971), Dumont et al. (1975), and Bottrell et al. (1976). The ln dry weight was regressed against ln length as described below.

Drying and Weighing of Zooplankton

In August and September 1980 and July 1981, zooplankton collected from Lakes 114, 223, 226, 239, 240, 302, and 382 at ELA were brought live to the field laboratory. Organisms were killed by exposure for 3–7 min to lake water at 60°C. Groups of individuals of the same species and life stage and approximately the same size were measured for length. Only organisms without eggs, embryos, or ephippia were selected. These groups were placed on predried and preweighed aluminum foil discs (0.66 cm in diameter) held on porcelain depression plates, dried at 60°C for 24–26 h, then weighed to the nearest μg. The foil discs and zooplankton remained on the hot porcelain plates until weighing so that the opportunity for the dried organisms to pick up moisture from the air was minimized.

The aluminum discs weighed 1622–1709 μg, or about 10–25 times that of the dried zooplankton. Weights of 10 discs dried and weighed three successive times were reproducible to ± 4 μg (coefficient of variability = 0.09 to 0.75%).

Calibration of the Geometric Formulae

In order to calibrate this new method we tested it against the accepted method of drying and weighing described above. Using regressions of weight on length, i.e., data arising from use of the original geometric formulae, we calculated dry weights per individual for organisms of the same lengths as those dried and weighed but whose width and depth measurements varied. These calculated dry weights per individual were averaged. If the calculated dry weight was different from the actual dry weights by more than 10%, the formula was altered and the weight–length regressions recalculated. For example, the formula originally chosen for *Cyclops bicuspidatus thomasi* adults resulted in calculated weights 1.1 to 1.3 times higher than directly obtained values for this organism, and for *Mesocyclops edax* the values were too low by about 50%. Therefore, the formula for copepodid and adult *C. b. thomasi* was changed to $4/3\pi abc$ and that for *M. edax*, to 4/3

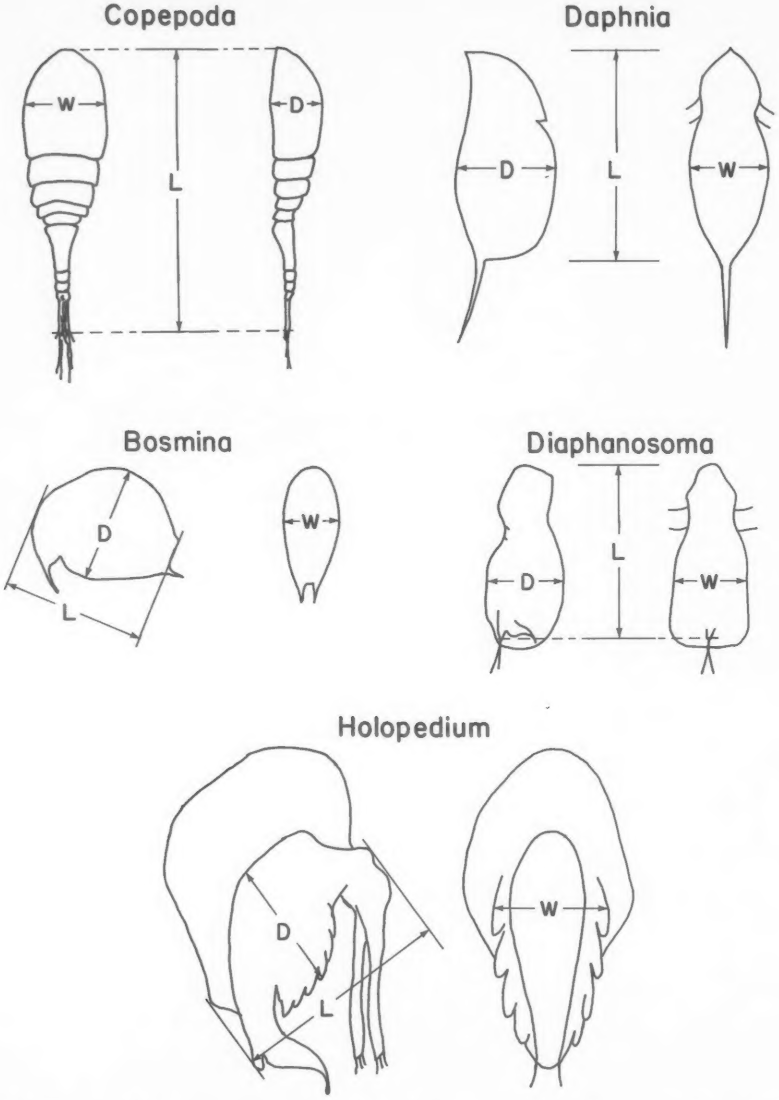

FIG. 1. Axes of measurements on copepods and cladocerans. L = length, W = width, D = depth.

$\pi ab1.5c$. These latter formulae reflect visible differences under the microscope between the species in the bulk of their appendages. The formulae for naupliar copepods and for other copepodid and adult copepods were not changed from those given above.

Using the initial formulae, the ratio of actual dry weights : calculated dry weights were 0.52 to 0.59 for *Bosmina longirostris*; 0.45 to 0.82 for *Daphnia galeata mendotae*; 0.27 to 0.35 for *D. catawba*; 0.41 to 0.50 for *Diaphanosoma birgei* and 1.22 to 5.84 for *H. gibberum*. To improve the agreement, the formulae for cladocerans were modified: for *B. longirostris*, *D. birgei*, and *Daphnia* except *D. catawba*, $4/3\pi a0.5bc$; for *D. catawba*, $4/3\pi a0.3bc$; and for *H. gibberum*, $4/3\pi ab1.5c$ for individuals longer than 1 mm and $4/3\pi ab3.0c$ for individuals shorter than 1 mm.

The probable reason for the non-ellipsoidal shape of *Daphnia*, *Bosmina*, and *Diaphanosoma* was the gaping of valves which occurred as a result of preservation. This phenomena was noted by Haney and Hall (1973). In contrast to other cladocerans, the simple ellipsoid underestimated the weight of *Holopedium* because the measurement did not include its sheath. It is probable that the gelatinous sheath contributes to the dry weight proportionately more in small than in large specimens. Therefore, for better agreement with actual dry weights, different formulae were used for organisms of different size.

Good agreement was achieved between directly measured dry weights (Table 1) and the dry weights calculated using the final geometric formulae (Tables 2–4). For those cladocerans represented over at least a two-fold length range, i.e.,

TABLE 1. Comparison of dry weight per individual obtained by direct drying and weighing of planktonic Crustacea (*A*) with calculated dry weights (*B*). The *B* values were calculated from the average length of the dried organisms using length–dry weight relationships developed for L223 zooplankton. The zooplankton were dried groups of *N* individuals with average length, *L*.

Species	Lake of origin	Average length ± SE mm (*L*)	Sex	*N*	Direct dry weight μg (*A*)	Calculated dry weight μg (*B*)	*B*/*A*
Copepoda							
C. b. thomasi	239	0.954 ± 0.010	M	20	1.85	1.80	0.98
	223	1.006 ± 0.010	M	20	2.98	2.22	0.74
	226	1.068 ± 0.003	F	20	2.75	2.77	1.01
	302	1.083 ± 0.004	F	30	3.52	2.93	0.83
	239	1.109 ± 0.010	F	20	3.47	3.24	0.94
	239	1.109 ± 0.010	F	20	3.50	3.25	0.93
M. edax	302	0.944 ± 0.003	M	29	3.36	3.19	0.95
	226	1.323 ± 0.004	F	12	7.33	8.94	1.22
	226	1.339 ± 0.003	F	12	9.08	9.27	1.02
	302	1.357 ± 0.008	F	15	9.92	9.66	0.97
D. minutus	114	0.949 ± 0.005	M	15	3.00	2.45	0.82
	239	0.989 ± 0.009	F	20	1.96	2.74	1.40
	302	1.038 ± 0.003	F	21	2.85	3.10	1.09
	114	1.093 ± 0.007	F	15	3.97	3.55	0.97
E. lacustris	226	1.496 ± 0.005	M	7	11.43	11.76	1.03
	226	1.582 ± 0.007	F	10	13.8	13.50	0.98
	302	1.777 ± 0.020	F	7	20.2	18.03	0.89
Cladocera							
B. longirostris	223	0.462 ± 0.003		50	1.04	0.90	0.86
	223	0.466 ± 0.003		50	0.95	0.92	0.96
D. g. mendotae	240	0.999 ± 0.018		10	4.8	2.96	0.62
	239	1.026 ± 0.017		20	4.6	3.20	0.70
	382	2.166 ± 0.020		20	21.94	24.19	1.10
D. catawba	223	0.878 ± 0.006		32	1.91	1.73	0.90
	223	1.265 ± 0.007		19	6.32	5.36	0.85
	223	1.502 ± 0.006		13	8.15	9.13	1.12
	223	1.620 ± 0.008		10	10.4	11.56	1.11
D. birgei	240	0.497 ± 0.004		31	0.32	0.37	1.16
	240	0.682 ± 0.004		38	1.16	1.04	0.89
	240	0.699 ± 0.004		28	1.29	1.12	0.87
	239	0.733 ± 0.012		10	1.72	1.32	0.77
	240	0.812 ± 0.003		30	1.80	1.82	1.01
	240	0.882 ± 0.005		32	1.92	2.37	1.23
	240	0.920 ± 0.018		20	2.70	2.81	1.04
	382	1.043 ± 0.019		40	3.26	4.32	1.33
H. gibberum	114	0.523 ± 0.007		10	4.5	2.22	0.49
lengths <1mm*	223	0.792 ± 0.009		15	4.67	6.65	0.70
lengths >1mm**	223	1.067 ± 0.012		15	7.6	7.36	0.97
	223	1.363 ± 0.016		13	14.1	14.40	1.02
	302	1.385 ± 0.007		8	15.5	14.98	0.97
	223	1.616 ± 0.039		10	21.47	23.19	1.08
	223	1.670 ± 0.021		8	20.3	25.10	1.24

* $4/3\pi ab3.0c$

** $4/3\pi ab1.5c$

D. catawba and *D. birgei*, agreement between calculated and actual dry weights was good over the entire size range. Agreement was less good for *D. g. mendotae* and for small *H. gibberum*.

Weight–Length Relationships

Because cladocerans form a continuous size distribution without morphological differentiation into instars, calculated

TABLE 2. Measurements and calculated dry weight biomass per individual of species and life stages of calanoid copepods from Lake 223. *N* is the number of individuals measured. Length is the longest dimension along the anterior–posterior axis, width is the lateral dimension, and depth is the dorso-ventral dimension. *a*, *b*, and *c* are the semi-axes of length, width, and depth, respectively. Values are means ± SE.

Species/life stage		*N*	Average length µm	Average width µm	Average depth µm	Formula	Average dry weight of individuals µg
Diaptomus minutus							
Nauplius 1		10	132.2 ± 0.9	69.4 ± 0.6	65.2 ± 0.8	$4/3\pi ab1.5c$	0.033 ± 0.001
Nauplius 2		10	153.4 ± 1.9	80.2 ± 1.1	77.5 ± 1.3	$4/3\pi ab1.5c$	0.052 ± 0.002
Nauplius 3		10	192.4 ± 2.8	93.2 ± 1.1	86.0 ± 1.0	$4/3\pi ab1.5c$	0.085 ± 0.003
Nauplius 4		10	224.5 ± 1.1	106.2 ± 0.9	97.7 ± 1.4	$4/3\pi ab1.5c$	0.128 ± 0.002
Nauplius 5		10	257.0 ± 2.6	114.2 ± 1.2	107.6 ± 1.5	$4/3\pi ab1.4c$	0.163 ± 0.005
Nauplius 6		10	291.5 ± 1.9	119.9 ± 1.3	123.1 ± 1.1	$4/3\pi ab1.25c$	0.197 ± 0.003
Copepodid 1		20	415.1 ± 3.2	127.3 ± 1.3	115.5 ± 1.5	$4/3\pi ab1.25c$	0.279 ± 0.006
Copepodid 2		20	517.4 ± 2.3	138.2 ± 1.8	126.7 ± 2.0	$4/3\pi ab1.25c$	0.416 ± 0.010
Copepodid 3		30	606.1 ± 7.7	172.0 ± 2.1	151.4 ± 2.9	$4/3\pi ab1.25c$	0.730 ± 0.025
Copepodid 4		30	771.7 ± 13.7	214.2 ± 5.3	192.2 ± 4.3	$4/3\pi ab1.25c$	1.500 ± 0.097
Copepodid 5	female	15	878.9 ± 26.7	234.5 ± 9.0	219.4 ± 8.0	$4/3\pi ab1.25c$	2.174 ± 0.237
	male	25	836.3 ± 16.8	227.8 ± 5.8	211.9 ± 6.0	$4/3\pi ab1.25c$	1.908 ± 0.126
Adult Copepodid	female	72	945.5 ± 7.9	255.3 ± 2.5	224.7 ± 2.4	$4/3\pi ab1.25c$	2.514 ± 0.064
	male	50	853.0 ± 5.6	231.0 ± 2.5	200.5 ± 2.0	$4/3\pi ab1.25c$	1.822 ± 0.040
Epischura lacustris							
Adult Copepodid	female	10	1854.4 ± 49.1	552.0 ± 14.0	449.6 ± 6.2	$4/3\pi ab1.25c$	21.277 ± 1.277
	male	10	1636.0 ± 41.8	470.4 ± 10.9	393.6 ± 7.9	$4/3\pi ab1.25c$	13.979 ± 0.754

dry weights were regressed against length. The relationship between body length and dry weight was assumed to be of the form

$$(1)\qquad \ln w = \ln a + b\overline{\ln L}$$

(McCauley 1984) where $\ln w$ is the natural logarithm of the dry weight in µg; $\ln a$, an estimate of the *y*-intercept; *b*, an estimate of the slope; and $\overline{\ln L}$, the mean of the log-transformed length measurements in mm. The slope of each regression was expected to approach 3 to reflect the increase in weight with increase in length which occurs in most animals (Peters 1983). All data were initially transformed by using natural logarithms in order to normalize the distributions and reduce inequality among variances (Persson and Ekbohm 1980; Prepas 1984). Estimates for $\ln a$, *b*, residual mean square (RMS), $\overline{\ln L}$, and $s^2_{\overline{\ln L}}$ (variance of mean $\overline{\ln L}$) were calculated according to standard statistical methods. Weight–length relationships were also calculated for naupliar, copepodid and adult life stages of copepods and for combined data from these groups to obtain regressions over larger ranges in length.

Weight–length relationships are used in turn to predict the dry weight of organisms of known lengths by converting the average length to a natural logarithm ($\overline{\ln L}$) and inserting it into Equation (1). Values for $\ln a$ and *b* are given in Table 4. The expected unbiased average weight (µg) for individuals in the group is then calculated from:

$$(2)\qquad w = e^{(\ln w + s^2_{\ln w}/2)}$$

where $s^2_{\ln w}$ is the variance of $\ln w$. This statistic is calculated as:

$$(3)\qquad s^2_{\ln w} = RMS\left(n^{-1} + \frac{(\overline{\ln L} - \bar{X})^2 - s^2_L}{s^2_{\bar{X}}\,(n-1)}\right) + b^2 \cdot s^2_L$$

where n is the number of paired observations used to derive the regression, $\bar{X}$ is the mean of $\overline{\ln L}$ measurements from the regression, $s^2_{\bar{X}}$ the variance of $\bar{X}$, and s^2_L the estimated variance of $\overline{\ln L}$. Inclusion of this variance corrects for skew which arises when logarithmic values are converted to arithmetic units. These variance and confidence intervals around the expected weight can be calculated according to equations in Persson and Ekbohm (1980, their tables 4 and 6) and McCauley (1984). Residual mean square (RMS) is presented in Table 4. It should be noted that the error represented by RMS in our method arises from the process of measuring zooplankton dimensions under the microscope instead of by weighing using a balance as is the usual case for weight–length regressions. We dried and weighed *D. birgei* of varying lengths (Table 1). The weight–length relationship obtained from these "direct" data had a RMS of 0.0307 which is in the range of RMS values reported in Table 4 for the "indirect" weight–length regressions. Therefore, we use our weight–length regressions for the calculation of average dry weight in the same manner as if they were based on direct weighings.

Assessment of the Method

The use of three-dimensional measurements and simple geometric formulae to calculate dry weight biomass depends on two assumptions. In converting volume to wet weight we have assumed that 1 mm^3 of animal biomass has a density of one, although organisms are clearly slightly heavier than water. We have further assumed that dry weight is a constant fraction of wet weight over all species. Nauwerck (1963) concluded that the dry weight of zooplankters lies between 5 and 10% of wet weight. Conversion factors reported by Bottrell et al. (1976) varied depending on the size of the organism, but most estimates for small zooplankton were under 10%. Schindler and Novén (1971) used 5% to convert volumes of zooplankton they measured to dry weight and 6% for converting wet weight values taken from the literature. K. Patalas (Freshwater Institute, pers. comm.) uses 7% of an average value for this factor. We have adopted this latter value of 7% for ELA zooplankton.

TABLE 3. Measurements and calculated dry weight biomass per individual of species and life stages of cyclopoid copepods from Lake 223. *N* is the number of individuals measured. Length is the longest dimension along the anterior–posterior axis, width is the lateral dimension, and depth is the dorso–ventral dimension. *a*, *b*, and *c* are the semi-axes of length, width, and depth, respectively. Values are means ± SE.

Life stage	Species	*N*	Average length µm	Average width µm	Average depth µm	Formula	Average dry weight of individuals µg
Nauplius 1	*	10	89.6 ± 2.3	49.6 ± 1.6	36.8 ± 2.4	$4/3\pi ab1.5c$	0.009 ± 0.001
	**	10	110.1 ± 1.1	71.9 ± 1.7	50.7 ± 0.3	$4/3\pi ab1.5c$	0.022 ± 0.001
Nauplius 2	*	10	102.4 ± 4.3	49.2 ± 2.4	49.6 ± 1.6	$4/3\pi ab1.5c$	0.017 ± 0.002
	**	10	128.4 ± 1.5	84.4 ± 1.4	57.8 ± 1.2	$4/3\pi ab1.5c$	0.034 ± 0.001
Nauplius 3	*	10	126.4 ± 3.7	68.8 ± 2.4	64.0 ± 0.0	$4/3\pi ab1.5c$	0.031 ± 0.002
	**	10	152.4 ± 1.6	94.0 ± 1.4	64.4 ± 1.5	$4/3\pi ab1.5c$	0.051 ± 0.002
Nauplius 4	*	10	136.0 ± 3.6	82.6 ± 1.8	62.4 ± 1.6	$4/3\pi ab1.5c$	0.039 ± 0.002
	**	10	174.2 ± 1.4	102.6 ± 1.4	74.2 ± 1.3	$4/3\pi ab1.5c$	0.073 ± 0.002
Nauplius 5	*	10	152.0 ± 4.3	84.8 ± 2.4	65.5 ± 1.6	$4/3\pi ab1.4c$	0.044 ± 0.003
	**	10	198.1 ± 1.9	114.1 ± 1.5	81.9 ± 0.8	$4/3\pi ab1.4c$	0.095 ± 0.002
Nauplius 6	*	10	154.4 ± 2.4	87.0 ± 2.1	65.0 ± 0.8	$4/3\pi ab1.25c$	0.040 ± 0.002
	**	10	242.2 ± 3.2	126.9 ± 1.7	89.7 ± 0.7	$4/3\pi ab1.25c$	0.127 ± 0.003
Copepodid 1	*	10	228.8 ± 2.4	83.2 ± 4.0	68.8 ± 4.2	$4/3\pi ab1.25c$	0.061 ± 0.006
	**	35	362.9 ± 6.7	139.8 ± 2.6	88.8 ± 1.7	$4/3\pi abc$	0.177 ± 0.006
Copepodid 2	*	10	290.4 ± 8.4	99.5 ± 4.1	72.8 ± 2.5	$4/3\pi ab1.25c$	0.098 ± 0.009
	**	32	471.1 ± 9.0	164.4 ± 2.6	110.1 ± 2.6	$4/3\pi abc$	0.320 ± 0.017
Copepodid 3	*	10	326.0 ± 4.6	116.4 ± 3.2	91.2 ± 4.6	$4/3\pi ab1.25c$	0.158 ± 0.010
	**	34	616.6 ± 11.5	203.5 ± 5.4	136.2 ± 4.3	$4/3\pi abc$	0.653 ± 0.035
Copepodid 4	*	10	371.2 ± 6.7	136.4 ± 5.4	89.6 ± 3.5	$4/3\pi ab1.25c$	0.211 ± 0.017
	**	45	774.0 ± 11.9	246.8 ± 4.2	183.2 ± 5.3	$4/3\pi abc$	1.333 ± 0.080
Copepodid 5	*	10	457.6 ± 12.7	156.8 ± 4.0	123.2 ± 3.4	$4/3\pi ab1.25c$	0.411 ± 0.027
	**	20	917.2 ± 17.4	280.4 ± 8.8	211.1 ± 8.6	$4/3\pi abc$	2.055 ± 0.179
Adult Copepodids							
Tropocyclops prasinus mexicanus	female	30	542.1 ± 5.2	172.5 ± 1.2	132.5 ± 1.5	$4/3\pi ab1.25c$	0.571 ± 0.014
	male	30	455.7 ± 4.9	133.5 ± 0.6	95.8 ± 1.7	$4/3\pi ab1.25c$	0.276 ± 0.007
Cyclops bicuspidatus thomasi	female	20	1027.6 ± 35.2	331.2 ± 14.5	212.1 ± 10.4	$4/3\pi abc$	2.918 ± 0.334
	male	15	884.7 ± 22.7	239.0 ± 6.1	149.9 ± 4.1	$4/3\pi abc$	1.193 ± 0.084
Mesocyclops edax	female	10	1309.2 ± 21.4	423.9 ± 6.2	286.4 ± 4.9	$4/3\pi ab1.5c$	8.785 ± 0.380
	male	9	780.4 ± 14.6	246.6 ± 3.1	170.2 ± 1.8	$4/3\pi ab1.5c$	1.807 ± 0.070

* *Tropocyclops prasinus mexicanus.*
** *Cyclops bicuspidatus thomasi.*

The reliability of our method depends also on the accuracy of our direct dry weights. The measured dry weights of *D. minutus*, *C. b. thomasi*, *M. edax*, *B. longirostris*, *D. catawba*, and *D. birgei* from ELA lakes are within or near values for organisms which occur in other North American lakes (Wilson and Roff 1973; Hawkins and Evans 1979; Makarewicz and Likens 1979; Yan and Strus 1980; Culver et al. 1985) except those of Roff and Kwiatkowski (1977). The latter authors report values for *D. minutus* and *H. gibberum* which are an order of magnitude higher than those of other workers. The average of measured dry weights of adult *E. lacustris* for ELA lakes (15.5 µg) is similar to that for this species found in other small lakes of the Canadian Shield (15.0 µg), but *Daphnia g.mendotae* of ELA lakes are heavier on average than those of these other lakes (Yan and Strus 1980). The range of lengths and dry weights of *H. gibberum* of Mirror Lake are 0.4–1.2 mm and 2.5–9.7 µg per individual (as estimated from the graphs of Makarewicz and Likens (1979)). The ranges of length and weight of ELA organisms are 0.52–1.67 mm and 4.5–21.5 µg, respectively. In the area where length measurements overlap, the agreement in weight is good; the average weight is 6.1 µg per individual for Mirror Lake organisms and

TABLE 4. Length–weight relationships of planktonic Crustacea in Lake 223 generated from measured lengths and calculated biomass. The number of observations (N), intercept ($\ln a$), the slope (b), 95% confidence intervals (C.I.), the average logarithm of the length in mm ($\bar{X}$), estimated variance of X, ($S^2_{\bar{X}}$), residual mean square (RMS), correlation coefficient (r), and range of length measurements (mm) are presented for the relationship $\ln w = \ln a \pm b\bar{X}$, where $\ln w$ is the logarithm of the dry weight (μg).

Species	N	$\ln a$	$b \pm$ 95% C.I.	$\bar{X}$	$S^2_{\bar{X}}$	RMS	r	Range length (mm)	Formula
Copepoda									
1. Nauplii									
D. minutus	60	1.2461	2.2650 ± 0.0722	−1.6053	0.0793	0.0061	0.99	0.128–0.300	See Tables 2 and 3
C. b. thomasi	60	1.2290	2.2520 ± 0.1011	−1.8209	0.0716	0.0108	0.99	0.108–0.256	
T. p. mexicanus	60	1.7143	2.5762 ± 0.2718	−2.0883	0.0491	0.0535	0.93	0.096–0.160	
2. Copepodids									
D. minutus	140	1.0783	2.7879 ± 0.0875	−0.4306	0.0708	0.0196	0.98	0.384–1.120	
C. b. thomasi	166	0.9032	2.7307 ± 0.0764	−0.5435	0.1143	0.0287	0.98	0.272–1.120	
T. p. mexicanus	50	1.2298	2.7965 ± 0.2437	−1.1230	0.0591	0.0430	0.96	0.224–0.496	
3. Adults									
D. minutus	122	1.0332	2.6060 ± 0.2769	−0.1002	0.0065	0.0157	0.86	0.728–1.104	
E. lacustris	20	1.4670	2.4741 ± 0.4966	0.5515	0.0114	0.0121	0.93	1.430–2.044	
C. b. thomasi	35	0.7606	3.9145 ± 0.3602	−0.0460	0.0238	0.0257	0.97	0.752–1.238	
T. p. mexicanus	20	1.7116	3.6663 ± 0.4495	−0.7724	0.0092	0.0080	0.97	0.422–0.582	
M. edax	19	1.3472	3.0087 ± 0.1490	0.0229	0.0735	0.0066	0.99	0.704–1.430	
4. Copepodids and Adults									
D. minutus	262	1.0542	2.7482 ± 0.0621	−0.2767	0.0679	0.0178	0.98	0.384–1.140	
C. b. thomasi	201	0.8370	2.6608 ± 0.0744	−0.4569	0.1341	0.0386	0.98	0.272–1.238	
T. p. mexicanus	70	1.0128	2.6360 ± 0.1714	−1.0229	0.0770	0.0398	0.96	0.224–0.582	
5. All life phases									
D. minutus	322	0.9212	2.1497 ± 0.0376	−0.5243	0.3383	0.0400	0.92	0.128–1.140	
C. b. thomasi	261	0.6154	2.0340 ± 0.5737	−0.7704	0.4500	0.0877	0.98	0.108–1.238	
T. p. mexicanus	130	0.4178	1.9913 ± 0.0798	−1.5146	0.3442	0.0737	0.97	0.096–0.582	
Cladocera									
1. Daphnia									
D. catawba	58	0.9455	3.1108 ± 0.0976	−0.1599	0.0833	0.0113	0.93	0.560–1.542	$4/3\pi a0.3bc$
D. g. mendotae	76	1.0797	2.7188 ± 0.0870	0.2637	0.2433	0.0352	0.99	0.368–2.429	$4/3\pi a0.5bc$
2. *H. gibberum*	43	1.8135	2.7406 ± 0.1080	0.3418	0.0300	0.0036	0.99	1.030–1.967	$4/3\pi ab1.5c$
	13	2.5058	2.6426 ± 0.2123	−0.3593	0.0421	0.0047	0.99	0.512–0.998	$4/3\pi ab3c$
3. *D. birgei**	85	1.2740	3.2454 ± 0.1908	−0.2940	0.1026	0.0800	0.96	0.390–1.318	$4/3\pi a0.5bc$
4. *B. longirostris*	88	2.4751	3.3614 ± 0.0946	−1.2210	0.0413	0.0082	0.99	0.204–0.492	$4/3\pi a0.5bc$

* We formerly called this species *Diaphanosoma brachyurum* (Chang et al. 1980; Chang et al. 1981; Lawrence 1980; Malley et al. 1982).

5.6 μg for ELA organisms.

Our method of estimation of zooplankton dry weight over the life cycle results in a pattern of growth in copepods which agrees well with that obtained by direct drying and weighing of instars (Ivanova 1973). Patterns of growth of *D. minutus* from Lakes 223 and 227 are compared with those exhibited by *Acanthodiaptomus denticornis*, *Arctodiaptomus bacillifer*, and *A. spinosus* in Fig. 2A and B. The patterns of increase of length of all these calanoids are very similar. The rate of weight increase in *A. spinosus* and in the *D. minutus* of Lake 223 diverges somewhat from the others in the late copepodid stages. In *D. minutus*, this divergence probably results from inclusion of more female than male Copepodid 4 (C4) and C5 in the sample. Except for *A. spinosus*, the weights of adult males of these calanoids are a relatively similar percentage of adult female weights (Fig. 2B). Importantly for the nauplii (N1–N6), which were not dried and weighed in this study, the agreement between our dry weight estimates and the published values is excellent (Fig. 2B).

In Fig. 2C and D, increases of length and weight in *C. b. thomasi* of Lakes 223 and 227 are compared with those of two other species of *Cyclops*, *C. strenuus* var *sevani* and *C. colensis* (Ivanova 1973). The pattern of weight increase among these organisms is very similar during naupliar development but the relative dry weights of copepodids tend to diverge. The difference between the rate of increase in length and weight of *C. b. thomasi* of Lake 227 and that of other species may be due to inclusion of varying proportions of male and female organisms.

In Fig. 2E, relative weight increase of *C. b. thomasi* of Lake 223 and *M. edax* of Lake 227 are compared to that in *M. edax* of Lake Severson, Minnesota (Comita 1972). Since the sex of *M. edax* of Lake Severson was unspecified, the weights of N1–6 and C1–5 are presented as percentages of the average weight of mature males and females. The weights of Lake Severson *M. edax* were calculated from calorific data. The

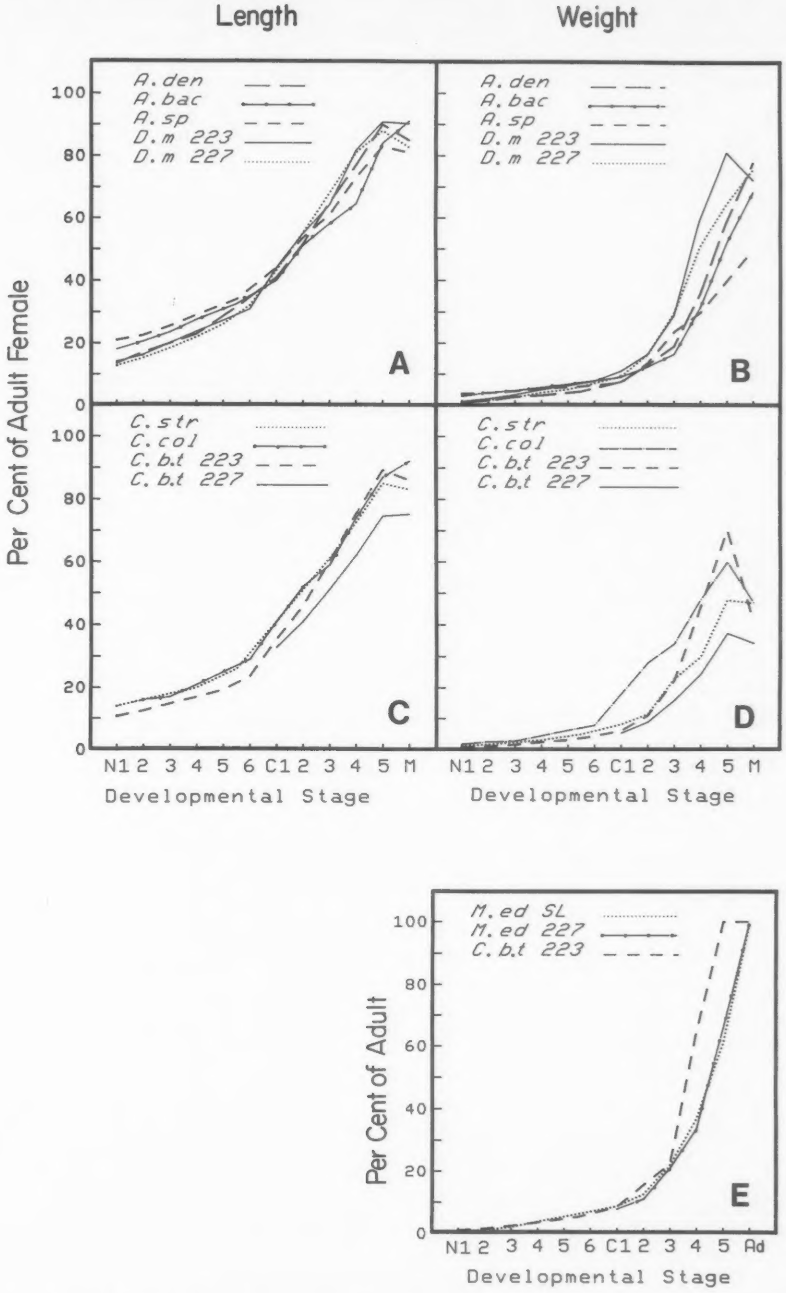

FIG. 2. Patterns of growth in length (A, C) and weight (B, D, E) in calanoid and cyclopoid copepods from naupliar (N1–N6) through copepodid (C1–C5) to adult male (M) stages are expressed as percentages of length and weight of adult females (A–D) or as percentages of the average (Ad) of the adult male and female weights (E). Growth patterns for *Acanthodiaptomus denticornis* (*A.den*), *Arctodiaptomus bacillifer* (*A.bac*), *A. spinosus* (*A.sp*), *Cyclops strenuus* var. *sevani* (*C.str*), and *C. colensis* (*C.col*) are from Ivanova (1973); that of *Mesocyclops edax* (*M.ed* SL) is from Comita (1972); and those for *Diaptomus minutus* (*D.m* 223, *D.m* 227), *Cyclops bicuspidatus thomasi* (*C.b.t* 223, *C.b.t* 227), and *M. edax* (*M.ed* 227) are from this paper and measurements on file at the Freshwater Institute.

pattern of growth and the calculated dry weight values for *M. edax* from both lakes are virtually identical. The pattern of weight increase in *C. b. thomasi* differs only slightly from *M. edax* in late copepodid stages.

Representative Data

Our method for calculating stage-specific dry weights of copepods may be used to estimate biomass from other lakes which contain different developmental stages when two conditions are satisfied. They are that the shapes of organisms are similar to those we treat and the method of preservation is the same. Similarly, the weight-length relationships may be used for estimating biomass of the copepods and cladocerans we treat where ranges of lengths of species or developmental groups are similar to ours.

Stage Specific Dry Weights of Copepods

The calanoid nauplii are essentially cylinders, slightly wider than deep (width : depth = 1.1), which proportionately elongate with each molt from length : width of 1.9 at N1 to 2.5 at N6 (Table 2). This 2.4-fold increase in length between N1 and N6 produces a 6.5-fold increase in dry weight per individual. The increase in length with stage is quite constant in the calanoid naupliar series at about 25–40 μm per molt (Table 2).

The calanoid copepodids are also cylindrical with width : depth of 1.1–1.15 but the molt from N6 to copepodid 1 (C1) produces a dramatic change in shape. Length:width becomes 3.0 to 3.4 and this remains relatively constant with development to adult forms where it is about 3.7. Increases in length with molt are much greater than in the naupliar series (Fig. 2A), 100–200 μm per molt. Over the copepodid series (C1 to C6) the increase in length is 2.3-fold for the female *D. minutus* but the increase in weight is 7 to 9-fold (Table 2). Calanoid females are 1.3 to 1.5 times heavier than males. These patterns of growth in length and weight are reflected in Fig. 2A and B.

In the cyclopoid nauplii, length : depth is the same as in the calanoids, but these nauplii are wider. Width : depth is generally 1.2 to 1.4. Proportions between the three dimensions do not change substantially from N1 to N6 (Table 3). Like calanoids, cyclopoids elongate during the molt to C1 and length : width becomes approximately 2.7. Cyclopoids remain wider than deep throughout the copepodid stages. Cyclopoid males are generally more slender than females. Males are 3.4 to 3.7 times longer than wide, whereas females are 3.0 to 3.2 times longer than wide (Table 3). Cyclopoids show greater differential between females and males in dry weight than calanoid copepods (Table 3, Fig. 2D). Females are 1.9 to 4.9 times heavier than males. *Mesocyclops edax* exhibits the greatest sex differentiation (Table 3).

Accuracy of length measurements is clearly more important for copepodids than for nauplii because of the large biomass of individual copepodids and because they are increasing in all three dimensions proportionately.

Comparable data to these shown for Lake 223 are available for the Lake 227 specimens from the Freshwater Institute.

Dry Weight–Length Relationships of Copepods and Cladocerans

Weight-length regressions generally have slopes close to 3, as expected from the power relationship between length and weight (Peters 1983). In cases where copepodids are not identified to stage, weight–length regressions (Table 4) may be used to obtain biomass estimates. The slopes of the weight–length relationships for various species of calanoid and cyclopoid copepods of both lakes are close to 3, with the exception of the slope for *C. b. thomasi* which is close to 4. The RMS of the regressions are low, but those of regressions formed for all life phases of a copepod species are generally higher than those formed for only part of the life cycle. The correlation coefficient, *r*, is above 0.9 for all regressions except those for *D. minutus* adults. The coefficient of determination, r^2, a measure of closeness of fit, ranges from 0.74 to 0.98.

The slopes of the regressions formed for cladoceran species are all near 3 (Table 4). The RMS for Cladocera are generally comparable to those for copepods when all life stages are combined. The correlation coefficients for cladocerans are all above 0.93. The statistic r^2 ranges from 0.86 to 0.98.

Weight–length relationships for a given species may differ through time and space because of differences in the sizes or the shape of organisms, in reproductive, or in nutrient state. Some of these factors tend to be cyclical with season. Frequently, similarities or differences in weight–length relationships from one study to another cannot be ascertained because of lack of statistical data necessary to calculate confidence intervals or to convert values from logarithmic to arithmetic units without skew.

We have identified several factors which cause the slope to deviate from the expected value of near 3. Inclusion of nauplier stages in the regression group flattens the slope (Table 4), apparently because nauplii elongate during growth whereas copepodids grow in three dimensions. Dumont et al. (1975) and Persson and Ekbohm (1979) observed this characteristic in regressions formed for *C. scutifer*. Also, in a regression model, it is assumed that the independent variable is evenly spaced throughout its range (Prepas 1984). This assumption was not satisfied for adult *T. p. mexicanus* or adult *C. b. thomasi*. Because of the differences in size of males and females, values of these two organisms clustered at either end of the length range. When immature male and female copepodids were included, the ranges were extended, the values more evenly spaced and the slopes approached 3.

Weight–length regressions have been generated for many species of zooplankton of European and North American lakes (reviewed by Bottrell et al. 1976 and McCauley 1984). Of these, four species are also found in Lake 223: *B. longirostris, D. g. mendotae, M. edax*, and *H. gibberum*. Few statistical data have been provided for any of these regressions, so that the accuracy of weight estimates when converted from logarithmic to arithmetic units is not known.

Application of the Method and Data

We report a method for the estimation of dry weight per individual for species of crustacean zooplankters common in ELA and representative data generated by this method. The dry weights for naupliar and copepodid stages may be used directly where copepods are identified to stage and subclass (or species) providing the body shape is similar; that is, the measurements of the individuals fall within the stated ranges (Tables 2 and 3). If these forms lie far outside the ranges, three axial measurements may be made and the dry weight calculated according to the geometric formulae (Tables 2 and 3).

Hawkins and Evans (1979) obtained a relatively constant weight for *D. minutus* from May to October but showed that the weight exhibits large, cyclic variation during a year period. In contrast, large variations in weight of *C. b. thomasi* occur during the course of the ice-free season (Hawkins and Evans 1979). Our average values for this organism (Table 3) may therefore be less applicable to other studies and we recommend the use of weight–length regressions for a more accurate estimate of dry weight. For less detailed zooplankton studies in which juvenile copepods are not identified to stage but the species is known, average dry weight biomass of the population can be calculated from the "all life phases" regressions in Table 4 providing that the average length lies within the range for the regression.

Our regressions for Cladocera produce estimates of dry weight much lower than those in some other studies. Use of our regressions for cladocerans of Lake 223 should be confined to ELA lakes or those of similar character. The major caution in applying our geometric formulae to other studies is for cladocerans. Our formulae are based on formalin-preserved material which may be subject to "ballooning" upon preservation. Cladocerans preserved, for example, by the "sugar-coated" method of Haney and Hall (1973) may require the generation of new geometric formulae closer to an ellipsoid.

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